

Politics, SALT and the Way Ahead

THE flurry of excitement last week when the Senate Foreign Relations Committee seemed, for a time at least, to espouse Senator Jackson's argument that the United States should not endorse the proposed agreement on the limitation of strategic missiles without reservation has its roots in politics, not in strategy or technology. That part of the SALT agreements which restricts the deployment of anti-ballistic missiles to two sites in the Soviet Union and the United States has had a surprisingly easy passage through the Senate, which is a striking proof of how widespread is the conviction that the deployment of ABMs can only make the strategic balance between East and West unstable. It must be assumed that the two super-powers have been prevented from agreeing that there should be no ABMs at all only by their irrational attachment to hardware, the only virtue of which is that it has been manufactured only recently—instant obsolescence, however desirable, is an affront to taxpayers. Or is it possible that the basis now proposed for the SALT agreement is welcomed by the military because it will allow both sides to keep their hands in with the technology of the ABM?

For some time, however, it has been increasingly clear that the agreement, which will for practical purposes prevent the further deployment of strategic missiles by the Soviet Union and the United States, would have a harder time in Congress, if only because the United States will be left with a numerically smaller striking force than the Soviet Union, at least during the five years for which the agreement is supposed initially to run. Senator Jackson could be counted on to protest. Given his record, Senator Jackson's demand that the second part of the SALT agreement should be coupled with a warning that a more permanent treaty would have to be based on a more obvious numerical equality was only a modest spanner in the works. The real trouble was the ambiguous reaction of the Administration, which appeared half-heartedly to welcome this intervention, either as a means of endearing itself to hawks or as a counter in the continuation of the SALT negotiations. It has only itself to blame, especially in an election year, that the Senate Foreign Relations Committee should have insisted on some clarification of where the Administration stands before agreeing to the second part of the treaty.

Missile counting is, of course, an inadequate guide to the strategic power of the two sides. The effectiveness of a missile force is a function not merely of its numerical strength but of its accuracy. For several years, the United States has been reasonably confident that the performance of its missiles is superior to that of Soviet missiles, and can only have been encouraged by what appears to have been the success of the development of the Poseidon submarine missiles and, perhaps to a lesser degree, by the development and deployment of multiple re-entry vehicles carrying separate warheads (MIRV). But there is nothing

in the SALT agreements to prevent the replacement of strategic missiles by more advanced types, which is no doubt why the two super-powers have settled on a five-year period for their treaty. And when Mr Melvin Laird, the Secretary of Defense, says (as he has been saying) that the new treaties make necessary an accelerated programme of research and development in strategic missiles, he is no doubt as much concerned with what will happen five years from now, if the agreement on missile restrictions lapses, as with the improvement of American strategic power in the meantime. This is why it is unfortunate that the Senate Foreign Relations Committee rose so quickly to the chance of twisting the Administration's tail. It would have been much more valuable to have used the occasion as one for reading Senator Jackson and his associates yet another lesson on the realities of strategic power.

But what will happen five years from now? Like most of the agreements on arms control so far reached, the SALT agreements are plainly limited in duration. They will last only if they are followed within a reasonable period by other more extensive agreements. Even the agreements on biological and chemical warfare, which appear to have a completeness of their own, could be undermined if lesser powers were persuaded that these were cheap ways of making a nuisance of themselves. The Non-Proliferation Treaty will become a nonsense if too many of the nations which have declined to sign it follow France and China in their assumption of the right to test nuclear weapons in the atmosphere. And the Non-Proliferation Treaty is even more vulnerable so long as the possibility remains that non-signatories may band together to do what the Soviet Union, Britain and the United States have forsworn. The chief threat to the permanence of the SALT agreements is precisely the qualitative improvement of missile performance which both signatories now anticipate. The most urgent question is, therefore, to know in what directions the second phase of the SALT negotiations, due to begin when the first treaties have been ratified, should be pointed.

The most obvious line of negotiation, an attempt to impose more serious restrictions on the number of strategic missiles deployed by each side, is probably a fruitless avenue. As things are, each side has enough striking power to deter the other so long as the missiles which embody it are immune from any pre-emptive attack.

If the permitted numbers were substantially reduced, however, say by a half, there would be much more scope for the Senator Jacksons of this world, in the Soviet Union as well as the United States, to argue about the arithmetical niceties of strategic equality. This is why an attempt to restrict the qualitative improvement of nuclear weapons is the most hopeful outcome of the second phase of SALT, and because it is unreasonable to expect that



the super-powers will at this stage in their affairs be prepared to exchange technical information about such things as the accuracy of rocket systems and then to forgo improvements, or to abandon the development of submarine defences (which are potentially but still remotely destabilizing), it is hard to see how the super-powers can now avoid biting the bullet of an extension of the Partial Test-Ban Treaty to underground tests as well as those in the atmosphere. To shrink from this issue will be to jeopardize the survival of the limitation on strategic striking forces beyond 1977.

Why is this such a contentious issue? The simple answer is that the military have not so far been seriously hampered in the continuing development of their strategic forces. Even under the new agreements, research and development on ABM systems will not, for example, be prevented. And Mr Laird will no doubt be granted his wish that research and development on the improvement of existing striking forces, which must necessarily involve the continued testing of warheads, will be accelerated. An agreement not to test warheads even underground would quickly put a stop to this, and would have the further advantage of persuading some of those nations which protest that the Non-Proliferation Treaty is unsymmetrical to sign on that dotted line. The objections to a comprehensive test-ban treaty are of two kinds. Some protest that no method of remote sensing could be relied on to detect all possible violations of a treaty, but with seismology continually improving, violations must surely be too great a hazard. It is also argued that if there were a ban on all kinds of testing, there would be a chance that one side or the other would be able, on the basis of laboratory experiments or theoretical calculations, to develop warheads and then weapons of which the other side was ignorant. But can this be a serious cause for anxiety? Generals are notorious for their unwillingness to trust in weapons which have not been tested, and there is in any case a chance that the nation embarking on the deployment of untested weapons would weaken and not strengthen its position. It is true that a comprehensive test-ban would be a serious impediment to the continuing growth of military technology, but is it not inevitable that some such restriction must be inseparable from effective measures of arms control? And surely it is not unreasonable that one sector of high technology should suffer, in order that the future of other sectors be better assured by a genuine restriction in the proliferation of nuclear weapons?

Whether the SALT negotiations can continue as a dialogue between the super-powers is another issue to be faced in the months ahead. Five years from now, China will be more than a power to be feared for its potential, and the degree to which the super-powers will be prepared to make deals between themselves will be compromised, to say the least of it, by their dispositions towards China. But it is also clear that a comprehensive test-ban would be a nonsense if France did not adhere to it, and following the recent tests of trigger devices in the Pacific Ocean, undertaken in the face of vociferous, if ineffectual, opposition, it is apparent that she too intends to join the super-powers in the nuclear club. The difficulty for the super-powers is that they will not be able to persuade the smaller fry to be reasonable without making concessions of a political character that will eventually undermine the strategic monopoly

which at present gives their bilateral talks a curious air of cosiness. Crudely, it would be necessary for them to stomach the idea that China would not agree to restrictions on strategic development unless it were assured of commensurable strategic power. There are also potential complications in the likelihood that British accession to the European Communities at the beginning of 1973 will revive earlier dreams of a European Defence Community.

But would it not indeed be disastrous if the list of strategic powers were to grow? This is the received doctrine. There must, however, be some level at which a comprehensive agreement on the disposition of nuclear striking forces would be preferable to the present arrangements under which the super-powers pretend that no other tensions than those between themselves are relevant to arms control.

100 Years Ago



THE *Journal of the Franklin Institute* calls attention to the following interesting lecture experiment:—It is well known that a light ball, as of cork, is sustained for some time near the summit of a vertical jet of water, issuing from an orifice of such a nature that the steadiness of the jet is maintained. The experiment becomes more striking when a vertical blast of air issuing from a large bellows is substituted for the jet of water, as in this case there is no apparent support for the ball, which comports itself in a very amusing manner. When a strong blast cannot be obtained, if a slender wire, about four times the length of the diameter of the ball, be passed through its centre, so as to have one-fourth of its length projecting from one end, and one half from the other, the balancing is more readily obtained, as any considerable change in the relative positions of the centre of gravity and the point of support is prevented by the movements of the rod.

WE learn from the *Journal of the Society of Arts* that the directors of the telegraphic lines of France have recognised the absolute necessity of improving the theoretical and practical knowledge of its clerks, and with this view elementary courses of telegraphy have been arranged in all the chief towns, at which the attendance of the *employés* is obligatory. In addition to this, a superior course of instruction is to be opened in Paris, and those clerks who have most distinguished themselves in the provinces will be sent to the capital to complete their instruction. The courses are all to commence on the first of October.

WE notice from the *Field* that on September 3 and 4 a sale of the surplus animals of the Zoological Gardens of Antwerp is to take place. The collection to be disposed of includes many of the rarer species of mammals and birds. In the former figures a young Indian rhinoceros, several species of antelopes, mouflons, and a male markhare ("which," says the *Field*, "offers a chance for any one desirous of increasing the size of our Welsh goats"). The birds include ostriches, several species of rare and new pheasants, and a considerable number of the rarer waterfowl, serpents, pythons, &c.

OLD WORLD

Australian and New Zealand Scientists Meet

from our Special Correspondent

Sydney, August 19

THE 44th congress of the Australian and New Zealand Association for the Advancement of Science, or ANZAAS as it is popularly known, which has occupied the University of New South Wales for the past week, has been by common consent an improvement on previous congress. The organizers are especially pleased with their innovation of giving over the afternoons to general symposia on topics such as world population growth, the problems of science writing and the future role of Australia in the world. The congress has even made a modest profit, with 3,500 registered participants paying \$A24 for attendance—a sum sufficiently large to cause the most surprising people to worry whether their payment will be deductible for income-tax purposes.

One theme running through the meeting has been the organization of science in Australia. Thus the President of the Congress, Dr R. G. Ward, who is general manager of planning and research with the Broken Hill Proprietary Company, provided a powerful criticism of the present practice of the Australian Government in channeling most of its expenditure on research and development through government laboratories, those of the Commonwealth Scientific and Industrial Research Organisation (CSIRO), the Department of Supply (responsible for defence research) and the Atomic Energy Commission. Taking the line that industrial research is not the cause of present social problems but an essential ingredient in their solution, Dr Ward argued that innovators in public laboratories are unable to maintain the daily contact with potential users which efficiency requires. In spite of the successes of government laboratories—and the CSIRO has an awesome reputation in Australia—he said that the return on the money spent in government laboratories is less than it might be “because of the absence of continuing contact between research worker and the industrial customer”.

This issue is likely to be an important issue in the next few months, at least if the Liberal Government survives the general election likely to be held in October. Dr Ward commended the work of the commission which is dispensing money for industrial development under the Industrial Development Grants Act of

1967. (A similar device was introduced in New Zealand in 1970.) Others are, however, less cheerful about the effectiveness of these expenditures. He also welcomed the Australian Government's decision to set up an Advisory Committee on Science and Technology as a step towards positive planning that might remove the “randomness and lack of coordination” which, in his view, at present characterizes Australian research and development. From a paper read to the congress on behalf of the Minister for Education and Science, however, it is clear that it will be some time before the advisory committee is formed and before it has been decided how tough a body it should be—by all accounts, the chairman of the new committee has not yet been chosen once and for all. It may be significant that Dr Ward, representing the largest company in Australia, argued for a transfer of funds from defence research to industrial research, on the grounds that “future pressures on our independence” are more likely to be economic than military.

Research and development in atomic energy is also likely to be a casualty of the wish to reap economic benefits from what the government spends, at least if declarations on this subject at the congress are to be taken seriously. Representatives of public utilities, the Atomic Energy Commission and academic economists agreed that the Australian Government has been right to postpone for a decade or so the plans to build nuclear power stations in Australia with which it has dallied for nearly twenty years. The essence of the case is that coal is still plentiful and cheap—and the search for oil and natural gas off the north and south coasts of the continent is beginning to yield discoveries which are likely further to depress the ambitions of the reactor builders. Technical and economic doubts about the reactor types at present available is also a deterrent. But there may be a killing to be made from the sale of uranium. Three officers of the Atomic Energy Commission, G. L. Miles, S. A. South and R. K. Warner, suggested that it would be possible to export a considerable quantity of uranium oxide a year without prejudice to the future needs of Australia itself. But there would be still greater benefits in exporting processed uranium—hexafluoride or even enriched uranium. According to the same argument, there has recently been “some foreign interest” in using

Australia as a regional base for uranium reprocessing and for the storage of waste products—perhaps the most practical suggestion so far for a use for the western desert.

Economists have been conspicuous at the congress (and there are historians as well) and have put some quantitative flesh on the bones of the old conundrum—how quickly and in what circumstances to exploit mineral deposits. In the past few years, issues like these have been increasingly argued in Australia, and in response to public anxiety about the degree of overseas ownership of the extractive industries (thought to exceed 50 per cent), a committee of the Commonwealth Senate has been set up to make recommendations.

At the congress this week, Dr Susan Bambridge of the Australian National University, Canberra, pointed to some strange anomalies in the rate at which the state governments of Australia levy royalties on the extraction of minerals. Queensland appears to be more generous to mining companies than most other states, chiefly because it wishes to encourage mining. But the result, according to Dr Bambridge, is that minerals are exported too cheaply and, indeed, she argued that royalties should be not merely made uniform but should be levied at a higher rate.

Other symposia at the congress have dealt with wider but less tangible themes. Contributions to discussions of demographic themes suggested that the old Australian belief that immigration is a benefit is quickly fading, although only Sir Phillip Baxter, until recently Chairman of the Atomic Energy Commission, seems to hold explicitly to the view that Australia should be kept as empty as possible against the time when the rest of the world will need it as a kind of Noah's ark. Professor J. C. Caldwell of the Australian National University, who considers that demographic transition will eventually bring a kind of stability to the population of the world, thought that world population might settle down somewhere between 11,000 million and 15,000 million (compared with the present 3,600 million) at some point in the second half of the next century.

At other symposia, architects wrung their hands over high-rise buildings for housing people, economists wrestled with the problems of reconciling public and private interests, especially where pollution is concerned, and there was a somewhat old-fashioned discussion of

the marihuana problem. According to Mr S. Hausman of New South Wales University, the drug in Australia is almost a drug on the market—the going price in Sydney appears to be between \$A25 and \$A30 an ounce, but bulk supplies are cheap, with consumers increasingly looking towards overseas supplies on the grounds of supposed quality. Mr Simon Hasleton of the University of Sydney reported at the symposium the results of surveys among university students which, among other things, demonstrate the now familiar if slight correlation between marihuana use and academic success, that roughly half of those who try marihuana never touch it again and that the incidence of regular marihuana consumption among university students appears to be about 7 per cent. Mr Hasleton thinks that there are in due course likely to be changes in the law, if only because of the difficulty of empanelling juries which do not include marihuana users.

VIVISECTION

Experiments Increase

MORE than five million experiments were performed on living animals during 1971, according to a white paper published last week by the Home Office. In total, 5,607,435 experiments were performed, 4.8 million of them without anaesthetics. Of the remainder, 0.5 million were carried out under anaesthetic but the animals were not killed before the anaesthetic wore off, and 11,000 were carried out to illustrate lectures.

Under the 1876 Cruelty to Animals Act experiments on cats, dogs, horses, asses or mules all require special certificates, and the returns reveal that 13,695 cats, 17,830 dogs and 549 equidae were the subjects of experiments last year.

The five million experiments were performed by 10,199 licensees in 596 registered establishments, but 5,439 of those who held licences during the year did not perform any experiments. More than one million of the experiments were carried out on behalf of the government or the Medical Research Council.

The white paper states that most of the experiments carried out without anaesthetic (these made up 86 per cent of all experiments undertaken) consisted of inoculations, external applications or stimuli, modifications in diet or environment, or administration of a pharmaceutical or biological product, followed in each case by observation of any effects. More than 400,000 animals were used in research on cancer, 250,000 for the purpose of public health or the diagnosis of disease. A further 1.3 million animals were used

in the testing of drugs, for use both in man and animals and most of these were the result of the 1950 and 1956 Diseases of Animals and Therapeutic Substances Acts which lay down mandatory tests for the standardization of sera, vaccines or drugs.

The white paper lists more than thirty cases of irregularities where licensees have gone beyond the authority granted by their licence, but no prosecutions resulted. The white paper states that the inspectorate set up under the act concluded that in none of the cases brought to its attention has there been any deliberate intention of evading the requirements of the act. In the course of the year the thirteen inspectors, nine of whom are based in London, made 3,650 visits, mostly without previous notice, to registered laboratories. In addition 203 visits were made to premises not included on the register.

The immediate reaction of the anti-vivisection societies to the 1971 figures was a howl of protest at the increase of

27,000 experiments on the 1970 figures. The National Anti-vivisection Society protested to the Home Office, drawing the department's attention to the Early Day Motion which has been placed before the House of Commons and signed by 137 members of parliament. The motion calls for a research institute to collate information about alternative research methods to vivisection—cell culture for example (see *Nature*, **234**, 318; 1971). The society hopes that if the motion attracts sufficient attention the Select Committee on Science and Technology might launch an inquiry into vivisection; its recommendation would then be put to the House. The society feels that it is making substantial progress towards reducing vivisection now that an appreciable number of MPs are taking up its case, and is further cheered by progress in the international front—the United Nations Social and Economic Council recently recognized the international association of anti-vivisection societies.

Advancing by Draws

THE three games of the Spassky-Fischer match played last week have all ended in draws. Spassky has been unable to reduce the three point deficit in the match and is now trailing by $6\frac{1}{2}$ to $9\frac{1}{2}$ points. Every draw brings Fischer's score closer to the winning total of $12\frac{1}{2}$ points.

The fourteenth game failed to show either player at his best. Some motiveless moves by Fischer (playing white in a Queen's gambit declined) culminated in an oversight which cost him a pawn. A few moves later, however, Spassky made a complementary error and the resulting rook and pawn ending was drawn.

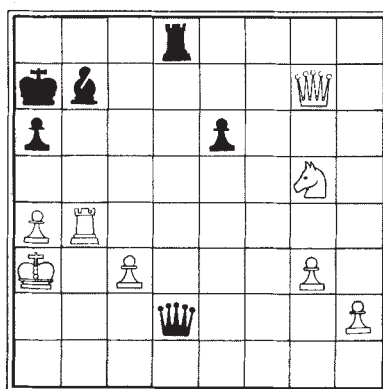
The fifteenth game was a most exciting one with both players striving hard for a win. Fischer chose a variation

of the Sicilian defence leading to cut and thrust play, and both sides, unusually, castled on the Queen's wing. Fischer sacrificed his King's knight's pawn for active pressure, and the game was evenly balanced until Spassky rashly took a second pawn on the 29th move in a desperate effort to win. The counter action by Fischer was swift and he must have missed winning by only a very small margin.

The diagram shows the position in the fifteenth game after white's 40th move. By playing 40... R-Q2 Fischer could have relinquished white's threat of mate and freed his pieces for the final attack. The move actually played precluded this possibility and Fischer had to be content with a perpetual check.

The sixteenth game was an accurately played but rather uninteresting draw. Spassky seemed well prepared for Fischer's exchange variation of the Ruy Lopez and never had any disadvantage in position.—J.P.

SPASSKY
WHITE



BLACK
FISCHER

White: Spassky, Sicilian Defence,		Black: Fischer Najdorf variation	
White	Black	White	Black
1 P-K4	P-QB4	23 P-K5	PxP
2 N-KB3	P-Q3	24 PxP	B-KR1
3 P-Q4	PxP	25 N-B3	R-Q1
4 NxP	N-KB3	26 RxR ch	RxR
5 N-QB3	P-QR3	27 N-KN5	BxP
6 B-KN5	P-K3	28 QxR	R-Q2
7 P-B4	B-K2	29 QxR	BxN
8 Q-B3	Q-B2	30 PxR	Q-N3 ch
9 0-0-0	QN-Q2	31 K-B1	Q-R4
10 B-Q3	P-N4	32 Q-R8 ch	K-R2
11 KR-K1	B-N2	33 P-QR4	N-Q6 ch
12 Q-N3	0-0-0	34 BxN	RxB
13 BxN	NxB	35 K-B2	R-Q4
14 QxP	QR-B1	36 R-K4	R-Q1
15 Q-N3	P-N5	37 Q-N7	Q-KB4
16 N-R4	KR-N1	38 K-N3	Q-Q4 ch
17 Q-B2	N-Q2	29 QxR	Q-Q7
18 K-N1	K-N1	40 R-QN4	Q-B8 ch
19 P-B3	N-B4	41 R-N2	Q-R8 ch
20 B-B2	PxP	42 R-R2	Q-B8 ch
21 NxBP	B-KB3	43 R-N2	Q-R8 ch
22 P-KN3	P-KR4		

Agreed drawn

NEW WORLD

Kennedy Floats Some Radical Changes

by our Washington Correspondent

SENATOR EDWARD M. KENNEDY'S National Science Policy and Priorities Act swept through the Senate last week with barely a ripple of interest expressed by the newspapers. The lack of public interest in the bill, however, belies its importance, for if passed by the House of Representatives—where its future is at best uncertain—it will lead to a fundamental shift in the activities of the National Science Foundation and cause a major change in science policy planning in Washington (see *Nature*, **237**, 306; 1972).

Designed to increase federal expenditure on science and technology directed towards solving domestic problems, the bill did not, however, survive its passage through the Senate entirely unscathed. An amendment proposed by Senator Peter Dominick and reluctantly accepted by Senator Kennedy axed the total expenditure proposed in the bill from \$1,800 million, spread over three years, to \$1,025 million. But Kennedy and his supporters did manage to defeat other amendments which would have weakened the bill, and it was finally passed by an impressive margin of 70 votes to 8, in spite of opposition from the Administration.

A central purpose of the bill is the establishment within the National Science Foundation of a Civil Science Systems Administration, which would support research and development into all kinds of urban problems such as health care delivery, transportation and pollution. Another part of the bill seeks to give the National Science Foundation authority for retraining scientists and engineers thrown out of work by cut-backs in defence spending. If passed by the House of Representatives, signed by President Nixon and fully funded—all of which are doubtful—the bill would add helpings of \$150 million, \$375 million and \$500 million to the foundation's plate in 1973, 1974 and 1975 respectively.

The Administration is unhappy about the bill for several reasons, chief of which is that it would involve the National Science Foundation in all kinds of applied research and development that is at present the responsibility of mission oriented agencies, and it would take the foundation even further from its original mission of supporting basic research. The large expenditures involved are also viewed with some concern in the White House. But under-

lying the Administration's attitude is the fact that it has itself been working for the past year on ways to stimulate utilitarian research and development, and it is naturally chary of having its thunder stolen in election year by a Democratic-sponsored measure.



Senator Edward M. Kennedy

All of these arguments surfaced during the debate on the bill in the Senate, chiefly through Senator Dominick, who called the bill unnecessary, but nevertheless voted for it. Dominick pointed to the fact that the Administration has increased expenditure on domestic science and technology by 65 per cent between 1969 and 1973, expanded the

problem-related research activities of the National Science Foundation and set in motion a number of conversion programmes for out of work scientists. "In short," he said, "there are numerous programs, both legislative and administrative, which focus on the same problems [as the Kennedy bill]". He also raised the question of the NSF's responsibilities under the bill overlapping with those of other agencies and expressed concern that the proposed Civil Science Systems Administration would duplicate work being carried out elsewhere.

Specifically, Senator Dominick took exception to two of the general principles outlined in the preamble to the bill and tried unsuccessfully to have them deleted. The two principles, which are among the most far-reaching items in the bill, are that expenditure on science should grow at least as fast as the Gross National Product and that expenditures on civilian research and development should be increased until they reach at least parity with defence research and engineering.

Dominick argued that GNP is not a measure of real growth, and that it would be unrealistic to tie research and development expenditures to it. Moreover, he suggested that it is the duty of the appropriations committees to determine expenditure levels, and such a provision would interfere with their work. As for increasing expenditure on civilian research until it reaches parity with defence spending, he pointed out that "the level of defense spending gives no indication of the optimum level of civilian research and development spending". Kennedy emphasized the need for some guaranteed increase in civilian research spending by pointing out that the proposed 1973 budget for federal expenditures on science and technology is 16 per cent below what it was in 1967 in real terms, and Dominick's amendment was defeated by 46 votes to 35.

Senator Dominick did, however, succeed in getting significant reductions in the funding proposed in the bill, chiefly for the Civil Science Systems Administration, which ended up with \$795 million in the final version of the bill, compared with \$1,200 million in Kennedy's version, and the retraining and conversion programmes which are set to receive \$200 million instead of the \$560 million proposed in the original version. Kennedy accepted these cuts reluctantly on the basis of a behind the scenes trade off in which Dominick withdrew a proposal to delete from the

CONGRESS

Critic with an Axe

A RESHUFFLE within the Senate Appropriations Committee following the death of Allen J. Ellender, former chairman of the committee, has given Senator William Proxmire the chairmanship of the subcommittee which deals with the budgets of NASA and the National Science Foundation. Together with the companion subcommittee in the House of Representatives, it is one of the most important committees dealing with science budgets. Senator Proxmire has for long been an ardent critic of some of NASA's programmes, particularly the shuttle, and his appointment has not been greeted with enthusiasm in the agency which he will oversee.

bill all funding for 1975, in return for cuts across the board for the three years encompassed by the bill. In any case, the bill contains only authorization requests, and the appropriations committees must agree to the expenditures if the bill is passed—it is very unlikely that they would give their blessing to the total amount.

What are the prospects that the bill will be passed by the House of Representatives before Congress disperses for the November elections? Much depends on how quickly it is dealt with in committee and on how long Congress stays in session. But the prospects are not good. For one thing, John W. Davis, chairman of the key subcommittee on Science, Research and Development, has been occupied with a tough primary election, and so the bill has languished in his committee since he introduced it at the beginning of July. And for another, the House of Representatives is already facing a backlog of business to be dealt with before it can break up, so it would be difficult to fit the bill into the schedule.

One aspect in its favour, however, is that it is likely to be cited by Senator McGovern as a vehicle for offsetting the effects of his proposed defence cuts, and there will thus be strong pressure on Democrats in the House to deal with it quickly. But, alternatively, the White House will want to keep it bottled up in committee, and is likely to put pressure on Republican members to stall it for as long as possible. In any case, if the bill is not passed this year, it will almost certainly pop up again next session.

AIR POLLUTION

Clean Air in Jeopardy

by our Washington Correspondent

ONE of the chief provisions of the Clean Air Act may be jeopardized by an acute shortage of low sulphur fuels in the United States. According to a study commissioned by the Environmental Protection Agency, supplies of low sulphur coal and oil will be grossly inadequate for each state to meet the primary air quality standards — which are designed to protect health—by 1975. Clean coal, particularly in the midwest, will be the biggest problem, but there is also expected to be a severe shortage of oil.

The standards, which were set by the EPA under the terms of the Clean Air Act, were designed to reduce sulphur emissions by 50 per cent between 1971 and 1975, and each state must detail how it intends to meet the standard. So far, the plans of 13 states have been approved by the EPA, and the others are under discussion.

The study, which was carried out by the Mitre Corporation of Bedford, Mass., consisted of adding up the quantities of low sulphur fuel required by each state in its plan for meeting the standards, and attempting to match it with the projected supply. The results are that in 1975, 592 million tons of low sulphur coal will be required, but only 268 million tons will be available. Similarly, 1,439 million barrels of low-sulphur oil will be required to meet the standards, but only 1,048 million will be available. The only fuel for which no acute shortage is predicted is natural gas.

Although there have been suspicions about the availability of low sulphur fuels in the US for some time, it was only about two weeks before the states were required to submit their implementation plans to the EPA that the Mitre Corporation's preliminary report exposed the gravity of the situation. That was in May, and the EPA did not make the study public until last week, after it had been leaked to Congressman Les Aspin of Wisconsin.

The agency clearly has a tough problem on its hands, for either it must relax the standards, at least in some areas, or find new sources of low sulphur fuels. The Mitre study examined essentially two approaches that the agency might take, one of which involves importing low sulphur oil to fill the gap and the other entails the modification of implementation plans for a few states, chiefly those in the midwest.

The first approach would result in imports of some 740 million barrels of oil a year, a quantity which would wreck the oil import quota system and disturb the balance of payments to the tune of \$3,000 million. The second approach would involve altering the implementation plans for those areas which will have little difficulty in meeting the standards, and use the available low sulphur fuels in the areas where air quality is worst. This approach, which the report calls "the least costly and lowest impact strategy", may, however, increase sulphur oxide pollution in some parts of the country.

The EPA's only public discussion of the study is in the *Federal Register* of May 31, 1972, some two weeks after the Mitre study was delivered to the agency. The EPA submission points out that "plans in the aggregate cannot be achieved by 1975 despite the best efforts of government and the private sector", and suggests that states should continue to develop their implementation plans on the assumption that the standards can be met. In the meantime EPA will be completing its studies of the aggregate situation and will suggest necessary changes to the states, and likewise modify federally promulgated SO_x regulations for achievement of the secondary

standards where appropriate. (The secondary standards are more stringent than the primary standards, and come into force in about 1977.) The EPA discussion concludes that "highest priority must be given to achieving the primary standards (health related) by the statutory deadline".

HEALTH RESEARCH

NIH Funds Vetoed

by our Washington Correspondent

THE financial bonanza that Congress had budgeted for the National Institutes of Health for the 1973 fiscal year has been vetoed by President Nixon. Living up to his frequently repeated promise to avert tax increases, Nixon has refused to sign the appropriation bill for the departments of Labor and Health, Education and Welfare — which includes the budget for NIH—because it added up to some \$1,800 million more than he had requested.

The veto could have been overridden by Congress if two-thirds of the members voted to do so, but supporters of the bill in the House of Representatives could muster only 203 votes against 171. The appropriations committees in the House and Senate must now go back to the drawing boards and come up with another bill that is closer to President Nixon's budget request. Congress had wanted to give the research institutes of NIH \$1,794 million—over \$300 million more than they received last year, and about \$200 million more than the Administration asked for.

In his message explaining his reasons for vetoing the bill, President Nixon called it "a perfect example of that kind of reckless Federal spending that just cannot be done without more taxes or more inflation, both of which I am determined to avoid" — holding down taxes is a bigger vote catcher than increasing expenditure on welfare. And he also warned Congress against sending the bill back with only token cuts. Such action, he said, would not satisfy his objections to the bill, and what he wants is a measure which involves total expenditures no greater than he originally requested. In the meantime, NIH must be content with a budget for 1973 which continues expenditures at the same level as 1972 (the 1973 fiscal year started on July 1). New programmes must therefore await passage of a more acceptable appropriations bill.

NEWS AND VIEWS

Reconstruction becomes Informative

THE reconstruction of a three dimensional structure from a set of two dimensional projections is of general interest in many scientific disciplines. The problem confronting the biologist, for example, is to determine the structure of an object such as a ribosome or a virus particle from a series of electron micrographs of that object rotated about an axis. The first question that arises is how many views are necessary in order to reproduce the object to a given resolution. Second, several methods of reconstruction have been proposed and criteria are needed for choosing the best one for a particular problem. Biological objects are inherently "noisy" because intensity variations in the final image are introduced by the method of specimen preparation, non-uniformity of staining and by the effects of the electron beam itself. Another important question is, therefore, how the method of reconstruction deals with the problem of noise. This question has been difficult to answer, for proponents of reconstruction methods have tended to evaluate them using model objects.

On page 435 of this issue of *Nature*, Klug and Crowther have attempted to answer these questions by presenting a theoretical treatment of the reconstruction of a general object from a set of projections. They approach the problem from the standpoint of information theory, because determining the structure of an object can be regarded as equivalent to recovering a signal by sampling the information transmitted by a noisy channel.

The theory is quite general and could, at least in principle, be applied to any reconstruction method. In their article they show how the number and distribution of available projections determine the resolution for an object of a given size. In order to apply the general theory, Klug and Crowther resort to the use of Fourier transforms and reciprocal space, and although this procedure may appear somewhat prejudicial to proponents of direct space methods, they defend it on the grounds of convenience; it can be illustrated by a simple optical analogy.

Any object can be considered as a linear combination of a set of orthogonal functions. It is the coefficients of these functions that are required to obtain the three dimensional structure. The projected density distribution can, of course, be expanded in terms of these basis functions, but the coefficients that are obtained in this series cannot be used directly to reproduce the object as the basis functions themselves have been modulated by the projection and reconstruction procedure. Klug and Crowther have shown that these operations of projection and reconstruction on each basis function reproduce the function but with a different weight, and it is this weighting factor that determines the maximum possible resolution of the object even if the inherent resolution of the micrographs is greater. The number of basis functions which have significant weight and therefore contribute to the final reconstruction is determined by the number of projections available, and the value of the weighting factor is determined by the geometry of the projections. A symmetric set of equiangular projections gives rise

to more significant weights than an asymmetric set.

It is at this point that the micrographs enter the picture. The coefficients of the basis functions derived from them include errors associated with the inherent noise in the object. If these errors become comparable to the weighting factor of the basis function, the inclusion of this function can produce spurious detail in the reconstructed object. This condition is more likely to occur for a highly asymmetric set of views. Conditions can thus be laid down for the determination of the number and distribution of projections necessary to obtain a given resolution for an asymmetric object of a particular size. The requirements in fact turn out to be the same as those previously obtained by Klug (*Phil. Trans. Roy. Soc.*, **B261**, 173; 1971) on the basis of simpler physical arguments.

This new theory would seem to provide objective criteria for the evaluation of reconstruction methods. Whether it will put an end to the debate about the relative merits of direct space methods and Fourier methods is less clear.

Bay of Fundy Tides

IN the article on page 441 of this issue of *Nature*, Garrett deduces a period of approximately 13.3 hours for the free mode of oscillation of the Bay of Fundy, forced by the lunar semi-diurnal tide (M_2) of the North Atlantic to produce some of the highest tidal ranges in the world. His analysis suggests that, in this mode, the whole Gulf of Maine is part of a single oscillating system containing the Bay of Fundy.

These deductions are theoretically interesting, especially in the light of some earlier work in which a different description of the development of the M_2 tide was put forward. The latter work argued in favour of a near resonant mode of oscillation in the Bay extending out to the edge of the continental shelf and following essentially the route of Jordan Basin and Fundian Channel; these calculations of the effects on the tides of a barrier placed across the upper part of the Bay suggested that a decrease in tidal amplitude would be expected at the position of the barrier. On the basis of Garrett's model, however, a slight amplification of the tides seems likely in such circumstances. All this has an obvious and important relevance to the problem of estimating the tidal heights which would be available for the operation of tidal power schemes in the head waters of the Bay.

Thus Garrett's contribution, apart from introducing a new technique in interpreting tidal data, reopens the discussion on the viability of obtaining tidal power from upper Fundy, in Minas Basin and in Chignecto Bay. In many respects this discussion closed in 1969 with the publication of the report of the Atlantic Tidal Power Programming Board on the feasibility of tidal power development in the Bay of Fundy. Garrett's article provides a new element of hope that the tides in the Bay may one day be harnessed to produce electrical power.

PROTEINS

Kinds of Kinase

from our Molecular Biology Correspondent

POSTSYNTHETIC modification of proteins is a widespread phenomenon, the best documented form of which is phosphorylation. This in turn has been defined in the most comprehensive detail in relation to its role in certain metabolic pathways, especially glycolysis. For other systems information is more fragmentary, though a little fact has often been made to go a long way by the strenuous exercise of imagination. In sorting out the role of cyclic AMP in regulating glycogen phosphorylation Krebs and his associates have written a new chapter of biochemistry, and this is now being extended to embrace lipolysis. Corbin *et al.* (*J. Biol. Chem.*, **247**, 3736; 1972) have isolated the cyclic AMP-dependent kinase from adipose tissue. Its resemblance to the skeletal muscle enzyme is very close. In particular, addition of cyclic AMP brings about dissociation of the protein into its regulatory and catalytic subunits, according to an equilibrium in which the nucleotide binds competitively to the former. The dissociated catalytic subunit then operates as a cyclic AMP-independent kinase, until inhibited by the addition of either its own or another (for example skeletal muscle) regulatory subunit. In terms of enzymatic properties and the stimulation of lipolysis the adipose and muscle kinases are essentially identical, and rat liver kinases of again very similar properties have also been described (Kumon *et al.*, *ibid.*, 3726).

The phosphorylation of all manner of proteins by kinases from different sources has been reported. Eukaryotic ribosomal proteins, for example, are phosphorylated by endogenous kinases. Traugh and Traut (*Biochemistry*, **11**, 2503; 1972) find that skeletal muscle kinase is capable of catalysing the phosphorylation of some, though by no means all, of the proteins of *E. coli* ribosomes. The origin of the specificity is obscure: four of the 30S subunit proteins and seven or more of the 50S react in varying degrees, and it is the same proteins that accept a label from isotopic ATP whether they are in the ribosome or in the free state. One protein is most prominently labelled in the large subunit. These findings may or may not have functional significance, for although a cyclic AMP-dependent kinase exists in *E. coli*, it has not been shown that it attacks ribosomes. In eukaryotic ribosomes, the issue seems to be clearer, and it has also been reported that a kinase actually resides in the ribosome itself. Jergil (*Eur. J. Biochem.*, **28**, 546; 1972) has been exercised to

show that this is a ribosomal protein, in the sense at least that it is not easily dissociated by low salt washing. Working with ribosomes from trout testes, he finds that the kinase is dissociated only at high salt concentration (0.6 M KCl), and that in its characteristics on an ion-exchange column it is distinct from the protamine kinase, which is present in high concentration in these cells. The ribosomal kinase will incorporate phosphoryl groups at serine residues of several ribosomal proteins and of protamines, as well as various histone fractions. There is a mild activation effect by cyclic AMP, of such a low order (10 to 25%), however, that Jergil suggests it might arise from a cyclic AMP-controlled kinase present only as a contaminant. What the substrate might be for which the new kinase lies in wait on the ribosome is an open question.

The modification *in vivo* of histones by phosphorylation and by other means has been recognized for some time, and attempts have been made to relate these effects to the interaction of the histones with the DNA, and thus claim them as transcriptional control mechanisms. Such notions have little basis in experiment, but it seems to be established that substitution of histone side chains is associated with particular stages of the cell cycle. In their most recent report, Louie and Dixon (*Proc. US Nat. Acad. Sci.*, **69**, 1975; 1972), working also on trout testes, describe the appearance of a family of ten electrophoretic zones in the arginine-rich histone f2a1 (or histone IV). Nine of these are shown by the incorporation of label to contain modified residues. Both phosphorylation of serines and acetylation of lysines proceed progressively, reach a maximum and then decline. In particular the tritium label initially appears predominantly in the diacetylated species, then in the tri- and tetra-acetylated components, and is only later transferred to the mono- and unacetylated fractions. Louie and Dixon favour the idea that the acetylation and phosphorylation serve to weaken the binding to the DNA, so that the histone is able to search for its correct position, apparently in a kind of annealing process. The deacetylation then liberates new cationic centres, which increase the strength of the electrostatic interaction, and lock the molecule in place. There follow some hypotheses about the specificity of function of different parts of the histone, together with the suggestion that acetylation may drastically change the α -helix-forming propensity of the highly charged N-terminal part of the chain, which one may take or leave according to disposition. Candido and Dixon (*J. Biol. Chem.*, **247**, 3868; 1972) have also found that acetylation in trout testes during spermatogenesis is in

fact much more extensive than, for instance, in the relatively dormant tissue, calf thymus. In three histone fractions there is acetylation at one or two sites (apart from f2a1, which reacts at four sites). In f2a2 the lysine primarily substituted has been identified, and occurs in a region closely homologous in sequence to a segment of f2a1.

The acetyl and phosphoryl groups of histone f3 of calf thymus have been studied by Marzluff and McCarty (*Biochemistry*, **11**, 2672; 1972), who have prepared three cyanogen bromide fragments, taken advantage of the presence of only two methionine residues, favourably spaced in the chain. The largest of those fragments has ninety residues, and contains all the modified side chains, comprising methyllysine, acetyllysine and phosphoserine. Lysine and serine residues in the other fragments are not modified. The authors further show (*ibid.*, 2677) that the modification is incomplete, two lysines being partially acetylated, one partially methylated and two serines partially phosphorylated. This clearly gives a considerable number of permutations for an extensive microheterogeneity.

(In my account of the thermolysin structure (July 14), specificity was wrongly defined. The enzyme hydrolyses the substrate on the imino side of hydrophobic residues.)

REINDEER

Successful AID

ON May 9 this year two reindeer calves were born to two cows which had been artificially inseminated 126 days previously. This is probably the first recorded case of successful artificial insemination of these animals, and the Reindeer Council of the United Kingdom says that mothers and calves are doing well.

The artificial insemination of two cows is the first step in a campaign to propagate reindeer herds in other unlikely parts of the world—as well as to introduce some fresh stock into the herd which has lived in the Cairngorms for the past ten years, without running into difficulties with Britain's quarantine regulations which tend to be fatal to reindeer. The Cairngorm herd has flourished and has even been profitable; by mid-1970 more than eighty reindeer had been bred in Scotland.

There is a tentative plan afoot to introduce reindeer into the Falkland Islands. The chief economic virtue of the animals seems to be that they turn lichen—an otherwise unused natural resource—into meat and leather, without damaging other parts of the natural environment such as trees. But flying reindeer to all the corners of the Earth

is costly; so with a view to at least halving their problem the Reindeer Council is investigating ways of storing reindeer semen. So far its efforts have met with no success, but at least it has established that the technique of artificial insemination works.

VARIABLE STARS

Smokescreen Stars

from a Correspondent

IN 1967 RY Sagittarii, one of the distinctive group of variable stars called the R Coronae Borealis type, underwent a large fall in brightness with a slow recovery during 1968–69, and this unique opportunity for a detailed study of its spectroscopic and photometric behaviour was fully exploited by astronomers at the Radcliffe, Cape, and Republic Observatories in South Africa (Alexander, J. B., Andrews, P. J., Catchpole, R. M., Feast, M. W., Lloyd Evans, T., Menzies, J. W., Wisse, P. N. J., and Wisse, M., *Mon. Not. Roy. Astron. Soc.*, **158**, 305; 1972).

Stars of the R Coronae Borealis type are luminous supergiants whose light outputs are a maximum and more or less constant for long periods; at irregular intervals, however, they become fainter by factors of up to 1,000 and then have spectra that are dominated by emission lines. Spectroscopic studies of R Coronae Borealis itself in the 1930s revealed an abnormally high abundance of carbon, which led to the suggestion by E. Loreta and J. A. O'Keefe that the irregular declines in brightness were due to the condensation of clouds of carbon particles around the star. More recently, modern spectroscopic studies have confirmed the high abundance of carbon and have also led to the inference that the hydrogen normally dominant in stellar atmospheres has been largely replaced by helium, as could be the case if a highly evolved star lost its outer envelope in a kind of strip-tease act to reveal the nuclear processed core. This result forms a link between the R Coronae Borealis stars and the so-called helium stars which have different looking spectra because they are hotter, but share the chemical composition and galactic distribution of the R Coronae Borealis stars; both types probably represent a short lived stage in the evolution of certain old stars with a mass comparable to that of the Sun.

Modern developments in infrared astronomy led to additional support for the soot cloud or smokescreen hypothesis in 1968, when it was shown by W. A. Stein, J. E. Gaustad, F. C. Gillett and R. F. Knacke of the University of California that when R Coronae Borealis was below maximum in the visible, the

deficit was made up by infrared radiation between 3 and 30 μm with a spectrum resembling that of a black body at about 900 K such as would be expected for a circumstellar dust cloud of the right size to produce the emission. Shortly afterwards T. A. Lee of the Goddard Institute for Space Studies, New York, and M. W. Feast, of the Radcliffe Observatory, Pretoria, reported similar results for the southern R Coronae Borealis variable RY Sagittarii which is of particular interest because it seems to undergo pulsations as well as its long term and irregular variation.

The South African photometric and radial velocity observations confirm the existence of cepheid-like pulsations with a period of 38.6 days, compatible with the properties of a helium star, and lead to a striking confirmation of the Loreta-O'Keefe hypothesis. In the two colour diagram in which the ultraviolet colour index $U-B$ is plotted against the ordinary colour index $B-V$, the pulsations cause the star to undergo a loop, which is steadily shifted to redder colours as the average light level declines, just as one would expect from differential absorption at various wavelengths caused by dust. Moreover, the dense "chromosphere", which causes the emission lines seen at minimum light output, is found to have continuous emission at shorter wavelengths than 4000 Å, which is attributed to the electron attachment continuum of CN, and thought to be

situated above the dust cloud. Eclipse models, in which the dust cloud merely covers the photospheric disk in the direction of the observer so as to reveal the chromosphere beyond the limb, like the Moon at a total eclipse of the Sun, are virtually ruled out by the infrared observations, even though the latest results of the University of California group (Forrest, W. J., Gillett, F. C., and Stein, F. A., *Astrophys. J. Lett.*, **170**, L29; 1971) imply some departure from spherical symmetry in the circumstellar dust cloud.

ION TRANSPORT

A Subject Matures

from our Photosynthesis Correspondent

IT would seem that the study of ion transport by plant cells has come of age. This was the feeling expressed by Professor J. Dainty (University of Toronto) at an international workshop on the subject held from July 18 to 21 in the Botany Department at the University of Liverpool. This meeting was initiated by Dr P. Anderson (University of Liverpool) and gave, for the first time, the opportunity for workers in this field to come together to discuss their research.

From the various papers and discussions it is now quite clear that plant and algal cells cannot be thought of as green squid axons or blood cells and that they are much more difficult experi-

Microtubules and Procollagen Transport

MICROTUBULES are implicated in the transcellular movement of procollagen in a study to be reported by Ehrlich and Borenstein in *Nature New Biology* (August 30). This investigation supports the hypothesis that microtubules are part of a common transport system for moving the products of secretory cells from their sites of synthesis to extracellular locations. Previous examples of microtubules having such a function have been suggested for the secretion of thyroid hormone, proinsulin and transmitter substances. In all these cases, colchicine and vinblastine, drugs which disrupt the structural integrity of microtubules, have been used as means with which to probe for microtubular function.

Ehrlich and Borenstein found that colchicine and vinblastine at concentrations comparable with those blocking mitosis can cause an accumulation of procollagen in organ cultures, and prevent its movement from its site of synthesis to its extracellular location in bone where it is converted to collagen. Other agents which disaggregate micro-

tubules, such as hydrostatic pressure and water containing 50 per cent D_2O , also have similar effects. Moreover, none of these agents or drugs inhibits processes already known to be required for procollagen transport. In contrast, dibutyryl cyclic AMP, a known stimulant of secretion in other cell types, also stimulates the procollagen to collagen conversion.

Cytochalasin, a fungal metabolite which is thought to interfere with processes depending on contractile microfilament systems, was also found by Ehrlich and Borenstein to have no effect on procollagen accumulation. They therefore exclude microfilaments from a role in procollagen transport. They suggest that the transport of procollagen involves a direct interaction with microtubules and the secretory substance or membrane bound vesicles containing it. There still remains, however, the possibility that the disruption of microtubules is indirectly affecting secretion, such as by altering cell shape, and future experiments must aim at eliminating this objection.

mental material than animal cells. Illustrations of this were provided by discussions about the difficulty of finding out which of the cell surface nucleosidases are associated with ion transport. Nevertheless Professor A. Kylin (University of Stockholm) presented evidence that ATPase stimulated by Na^+ and K^+ could be isolated from a variety of plant tissue.

Plant cells usually contain a large central vacuole, necessary for maintaining a rigid cell wall, which has often made accurate analyses of internal ion concentrations difficult. Dr A. Lauchli (Technische Hochschule, Darmstadt) showed, however, that electron probe analysis and other physical techniques offer hope that reliable values may soon be obtained.

Progress in these areas is bound to be slow; but substantial progress has been made in the elucidation of active ion movements. A joint paper by Dr J. Raven (University of Dundee) and Dr F. A. Smith (University of Adelaide) crystallized the ideas of many, and now it seems likely that the outer membranes of plant cells will have to be thought of as helping to regulate the cytoplasmic pH by pumping out H^+ . The proton pump seems to be electrogenic and Dr R. Spanswick (Cornell University) presented data for *Nitella translucens* to show that an understanding of the electrical properties of this pump can explain previously inexplicable information about plasmalemma conductance. Further work in this area is likely to be stimulated by the detailed analysis by Dr W. J. Vredenberg (Centre for Plant Physiological Research, Wageningen) of current-voltage characteristics of the plasmalemma of *N. translucens* and by his suggestions concerning the relationship of the capacity of the proton pump and the changes in the energy state of the cell.

The net uptake of Cl^- by plant and algal cells could well be associated with the pH regulating pump, but as yet there is no generally accepted mechanism. There is, however, according to Drs A. F. Hill and B. S. Hill (University of Cambridge), evidence that in the salt glands of *Limonium* active pumping of Cl^- is associated with ATPase activity.

The control of ion transport in higher plants seems set to be one of the next areas of intensive research. Dr M. G. Pitman and J. Cram (University of Sydney) set the scene with their survey of the problem. Abscissic acid is likely to be an important messenger for the control processes and Dr T. Collins (University of Liverpool) provided support for this from a comparison of the *in vitro* effect of the hormone and induced *in vivo* changes of ion content and root exudation. Future studies will be heavily dependent on information about *in vivo* hormone levels.

PARASITOLOGY

Use of Genetic Markers

from a Correspondent

WHEN parentage is in doubt (as in the offspring of multiple matings), it can often be established by the use of genetic characters as markers. A novel use of this technique to ascertain the parentage of offspring from multiple infections of hosts by hymenopterous (wasp) parasites is reported by Holmes (*Entomophaga*, 17, 79; 1972). Such multiple infection, in the absence of mechanisms which prevent it, occurs chiefly when the proportion of hosts attacked and the probability of local host extinction are close to their maximum values. The consequences of multiple infection are thus of crucial importance to the stability of the host-parasite system in terms of population dynamics and must be incorporated in any realistic model of this system.

It is known that female *Nasonia vitripennis* (Chalcidae) lay fewer eggs in hosts (housefly puparia) which have been parasitized previously, and that the proportion of male offspring increases in cases of multiple attack. Hymenoptera can control the sex ratio of their progeny; fertilized eggs become diploid females and unfertilized eggs produce haploid males. The sex ratio change observed in *N. vitripennis*, however, could have been the result simply of better competitive ability of male parasite larvae in conditions of food shortage.

Holmes used three pure parasite strains differing in eye colour, and exposed each host to a known sequence of single, isolated parasites. The duration of exposure was short enough to

keep the number of offspring below the maximum which could be supported by the host, thus rendering parasite deaths from competitive effects unlikely. The progeny of parasites which had attacked first, second and third could be identified from their eye colours. Holmes found an increase in the proportion of males resulting from each successive attack, as well as the expected decrease in number of offspring.

It is implicit in these findings that female *N. vitripennis* are able to discriminate, not merely between healthy and parasitized hosts, but between those which have been attacked once and those which have been attacked twice. The number of eggs laid and the proportion fertilized are varied accordingly. Experiments with this and other wasps have shown that a dramatic rise in the proportion of male progeny results when searching female parasites are allowed to interact with each other. Thus, in *N. vitripennis* there are at least two separate mechanisms which tend to increase the male/female ratio when there are high densities of parasites. This conforms with theoretical predictions. Parasites emerging from a single host usually mate with each other. In cases of single attack this amounts to complete inbreeding, and the optimum number of males is the minimum number necessary to fertilize all the females. Any additional males would be produced in lieu of females, thus reducing the total number of eggs laid. These additional males could only contribute to the spread of their parental genes by mating with females other than their sisters. Where previous parasitism is detected, the probability of such matings increases and, in consequence, so does the optimum proportion of males.

New Media for Affinity Chromatography

IN next Wednesday's *Nature New Biology* (August 30), Porath and Sundberg describe a new chemical scheme for the coupling of biological molecules to a column matrix. The use of this kind of procedure has become indispensable for the isolation from complex mixtures of substances of specific affinity for an available ligand. One of the limitations has been the difficulty of attaching a sufficient density of the ligand species to the matrix to achieve good yields, and there has thus been a search for new methods of activating the matrix material.

Porath and Sundberg show that the introduction of reactive hydroxylic groups offers many advantages. They used the phenolic hydroxyls of the polyhydric phenol, phloroglucinol, which is coupled to agarose beads by cross-linking with epichlorohydrin. The phenolic groups may then be activated to receive

the relevant ligand by way of its amino groups in any of the usual ways, for example with cyanogen bromide. Porath and Sundberg, however, offer a new suggestion, namely divinylsulphone. This chemisorbent is rapid and efficient, and in the system described led to an increased capacity over the cyanogen bromide-treated matrix by a factor of 2.5. The agarose beads become stiffer after this treatment and have good flow properties in columns.

The system also offers the advantage that the link joining the chemisorbent to the column matrix is quite long, which diminishes the likelihood of steric impedance of the binding sites. As a demonstration of their method Porath and Sundberg coupled soyabean trypsin inhibitor to agarose and used a 1.4×3.1 cm column of this material to catch trypsin. No less than 80 mg was recovered from a single experiment.

SYMBIOSIS

Temperate Cleaners

from our Marine Vertebrate Correspondent

SYMBIOTIC cleaning relationships between fishes were first explored and described in the 1950s when marine biologists using SCUBA diving gear were finally able to get on to something like equal terms with their subjects. A considerable literature has since developed around the subject, and it has become clear that many tropical marine fishes practise this form of symbiosis. Essentially it consists of a small species of fish, usually conspicuously marked or coloured, acting as a cleaner of parasites or fungal infections to larger, even predatory fishes, which allow it immunity. Most examples described so far have concerned fishes in tropical communities; observations on temperate cleaner fish are few, and mostly concern the Californian seniorita (*Oxyjulis californica*). Recently, however, observations on cleaner symbiosis have been made in temperate New Zealand waters, and A. M. Ayling and R. V. Grace have published (*NZ J. Mar. Freshw. Res.*, 5, 205; 1971) an interesting account of their findings.

Ayling and Grace have found that in the waters of north-east New Zealand there are no fewer than five species of fish which act as cleaners to other fishes. All are wrasses—a family much given to cleaning behaviour. All are distinguished by the possession of very obvious markings, such as would be easily recognized by other fishes. Four of them have one or more contrasting stripes on the body, and the juveniles of the fifth species, *Pseudolabrus miles*, have a black bar across the base of the tail fin (in this species only the juvenile fish act as cleaners). Such distinctive marks, known as "guild marks", are conspicuous features of tropical cleaner fishes.

In New Zealand waters the cleaner fish do not adopt fixed individual territories, as do most tropical cleaners, some of which can be immediately recognized by their attention-arresting behaviour on some conspicuous site, as well as by their guild mark. Instead, the New Zealand fishes, such as the young *Coris sandageri*, seem to have loosely defined cleaning stations which are inhabited by groups of fish, whereas others are solitary but non-territorial in their behaviour. None appeared to display conspicuously to attract customers, their cleaning reaction being released by the approach and behaviour of the other fish. Larger fishes were, however, approached by the cleaners. All fish, no matter what their size, seem to react in the same way to the cleaner's approach by stopping swimming and by spreading their fins. Depending on their centre of gravity they assume a

head-up or tail-up posture. The red mullets (*Upeneichthys porosus*) observed by the authors also often open their mouths wide when posturing to allow the cleaner fish to search for parasites, although actual entry of the mouth was only observed on one occasion. Then a young *Coris sandageri* removed a 2 cm long parasitic isopod from the mouth of a red mullet, and before eating it, disabled it by beating it on a nearby rock.

Study of the stomach contents of the five species of wrasse has shown that they are not completely dependent on ectoparasites as food. One, an undescribed species of *Halichoeres*, is nearly an obligate cleaner, only occasionally feeding on benthic crustaceans, but the others are facultative cleaners, much of their food being free-living crustaceans. In spite of this even the facultative cleaners are very active, and Ayling and Grace record one *Coris sandageri* which in 15 minutes cleaned twenty-one fishes belonging to four species.

Ayling and Grace's observations greatly add to knowledge of this kind of symbiosis in temperate waters, and confirm some of the general conclusions that had been postulated by others. The lack of display, gregarious behaviour, and in the main facultative cleaning are all typical of temperate cleaners. Cleaning symbioses in temperate waters are clearly less important for the health of the fish than in tropical

waters, presumably because ectoparasites, bacterial, and fungal infections are less prevalent in cooler waters than in the tropics. The work which Ayling and Grace have carried out has shown, however, that this behaviour can be more widespread in temperate regions than had been previously appreciated.

ATMOSPHERIC POLLUTION

Limestone Dust on Trees

from our Plant Ecology Correspondent

RECENT work on the effects of the accumulation of limestone dust on forest composition in southern Virginia highlights some of the pitfalls associated with studies of the effects of atmospheric pollution (Brandt, C. J., and Rhoades, R. W., *Environmental Pollution*, 3, 217; 1972).

Most of the evidence for effects of atmospheric pollution on natural ecosystems is of a circumstantial nature, and as yet there have been few experimental studies or long term documentation of vegetational changes in areas subject to such pollution. Undoubtedly much of the circumstantial evidence is valid and can be interpreted in terms of changes brought about as a direct consequence of pollution; a good example is the work on lichen distributions in relation to areas of high concentration of

SV40 DNA Synthesized Discontinuously

IN next Wednesday's *Nature New Biology* (August 30) Fareed and Salzman report a further step in their exemplary analysis of the replication, in monkey cells, of the covalently circular, double stranded and supercoiled DNA of Simian Virus 40. Last year Salzman's group described the isolation of the replicative intermediates of SV40 DNA which contain two forks, three branches, one of which is the supercoiled unreplicated part of the parental molecule, and no free ends. These structures, precisely those predicted by the Cairns model of replicating circular DNAs, resemble the replicative intermediates of the DNAs of *Escherichia coli*, phage lambda and colicin E1 and the latest finding of Fareed and Salzman, that SV40 DNA is replicated discontinuously, provides a further parallel with the replication of these prokaryotic DNAs.

When dividing *E. coli* cells are fed for a few seconds pulses of ³H-thymidine, the label is incorporated into short DNA strands, the so-called Okazaki pieces, which are then ligated to the nascent daughter DNA strands. In

other words the daughter DNA strands of *E. coli* are made as a series of discontinuous pieces. Pulse labelling monkey cells infected by SV40 for periods as short as 15 s is technically difficult but, as Fareed and Salzman have shown, by no means impossible, and during such short pulses label is incorporated into short DNA strands which sediment at 4S and have a molecular weight of about 50,000 daltons. As pulse chase experiments reveal, these 4S pieces of DNA are incorporated into the nascent daughter SV40 DNA strands; in other words apparently both strands of SV40 DNA are replicated from start to finish in the same discontinuous way in which *E. coli* DNA is replicated.

The significance of these findings is manifold. This is, for example, the first evidence of discontinuous synthesis of a very small circular DNA molecule and these observations prove that at least some DNAs in a eukaryotic cell environment are replicated by a mechanism essentially similar to that which seems, by all accounts, to prevail in prokaryotes.

sulphur dioxide (Hawksworth and Rose, *Nature*, **227**, 145; 1970). Great care must, however, be taken in interpreting evidence of this kind, for it falls far short of the goal of proving relationships between causes and effects.

Brandt and Rhoades content themselves with a careful but static description of forest composition in an area of limestone quarrying in the Appalachian Mountains and compare this with forests in similar regions beyond the influence of the quarrying. Undoubtedly the dust fall is very considerable in the test area (of the order of 400 tons km^{-2} month $^{-1}$) and one cannot deny the very real differences in forest composition and tree density between the two sites. It is, however, a very tenuous assumption that the rate of dust accumulation is the chief distinguishing feature of the sites and therefore the cause of vegetational differences. It is also questionable whether the two sites were identical before quarrying began just after the end of the Second World War.

Brandt and Rhoades may be justified in their assumption, because some of their data seem to support them. For example, some species, such as *Liriodendron tulipifera* (tulip tree) and *Acer saccharum* (sugar maple) are far more abundant as seedlings at the dusty site than in the "control" area. There are, however, factors other than dust which could equally well account for this difference; for instance, the proximity of seeding trees. Some canopy species of the dusty site do not appear to be regenerating efficiently; for example, *Tilia americana* (lime) and *Quercus velutina* (black oak) are present at seedlings but not as saplings. Brandt and Rhoades consider that this implies a failure of the seedlings to survive and establish themselves in the shrub layer.

Though this may be so, the only conclusion that can be fully supported by the data is that there has been a failure in seedling establishment or survival in the past. The causes of past failure may be totally unconnected with intensification of quarrying and it is entirely possible that the present generation of seedlings will survive.

The most unfortunate feature of this type of reasoning is that the conclusion reached may be correct. If the accumulation of limestone dust is indeed altering the course of forest succession, then it is a matter of interest, and possibly of concern, to those involved in environmental conservation. The publication of premature conclusions extrapolated from inadequate data can do nothing but harm to the academic status of environmental research. Conclusive results can only be obtained if the absolute necessity of long term studies is fully recognized.

Is the Troodos Massif of Cyprus Oceanic?

THE idea that the Troodos igneous complex of southern Cyprus evolved in an oceanic environment has been in the air now for more than twenty years. Even the more specific suggestion that the province is actually an exposed segment of ancient oceanic crust has been current for a decade; but anything resembling convincing proof has proved elusive. Thus the evidence from palaeomagnetic, petrological and magnetic field studies, and from seismic velocity measurements on the outcrops, is not conclusive, although the internal structure and gravity anomalies are at least consistent with an oceanic origin. The problem is a difficult one but the solution is important because a proven, sub-aerial occurrence of oceanic crust would offer direct access to a type of environment which plays a central part in the concepts of the new global tectonics.

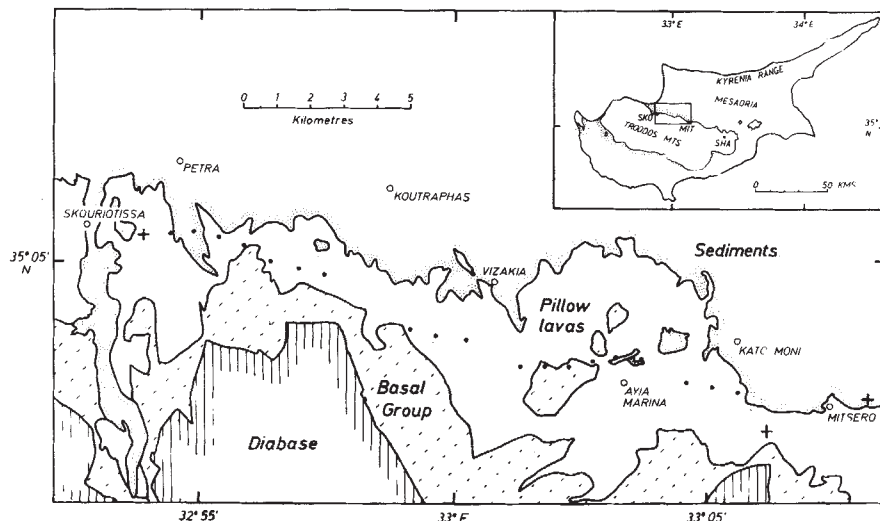
In next Monday's *Nature Physical Science* (August 28) Khan *et al.* report a further attempt to settle this question—an attempt which also proves inconclusive but which nevertheless adds weight to the oceanic crust hypothesis. What Khan and his colleagues have done is to carry out a seismic refraction study to determine the layering structure of the Troodos complex. The rationale behind the experiment is simple enough. Continents and ocean floors have quite different structures, the latter comprising three remarkably uniform layers (1, 2, 3) together with the upper mantle beneath. If it can be shown that the layering and seismic velocities of the Troodos complex resemble those of the present ocean floor rather than those of the continents, the case for an oceanic origin would be greatly enhanced.

Unfortunately, practical problems prevented Khan and his colleagues from fully realizing this aim. For one thing, the maximum shot size available limited the length of the refraction line to 20 km (between Skouriotissa and Mitero); but even had larger shots been

possible, poor transmission would have prevented a significant extension of the line. Nor were the travel-time curves easily interpreted. Thus although the first arrivals suggested the presence of two refracting discontinuities below the surface layer, no detector picked up first arrivals from the upper refractor from both ends of the line.

On the positive side, however, Khan *et al.* show that the surface layer (pillow lavas) is about 0.5 km thick and has an average seismic velocity of 3.25 km s^{-1} . This agrees well with velocities of 2.9 km s^{-1} and 3.7 km s^{-1} previously obtained for other Troodos pillow lavas. The apparent velocity of the second layer was found to be 5.10 km s^{-1} from the west and (less clearly) probably 5.28 km s^{-1} from the east—a difference which probably reflects the irregular form of the igneous units. First arrivals from the surface of the third layer were clearer, however, and were received from both shot points at detectors between 8 and 15 km from Skouriotissa. The velocity of the third layer was thus 6.38 km s^{-1} , and its depth probably 1.5 to 2.0 km.

Direct comparisons between these velocities and the velocities in oceanic layers are not possible because of differences in the confining pressure (layer 2 in the ocean floor is overlain by 4 to 5 km of sea water and a layer of sediment). Nevertheless, Khan and his colleagues equate their third layer (6.38 km s^{-1}) with layer 3 in the ocean floor (6.69 km s^{-1}), the velocity difference being reasonably explained on the basis of pressure difference. The two upper layers separated by the less clear refractor are then correlated with oceanic layer 2 (5.07 km s^{-1}). Thus, in general, the Troodos complex seems to be consistent with modern ocean floors, although, obviously, this interpretation would be more convincing had it been possible to extend the line to receive refractions from the Moho.



Three-dimensional Image Reconstruction from the Viewpoint of Information Theory

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Criteria are given for obtaining maximum information from a set of projections of a general object while preventing the introduction of spurious detail by effects which are beyond resolution.

THE problem of reconstructing an object from its projections arises in the derivation of the three-dimensional structure of a biological specimen from a series of electron micrographs. Various methods have been proposed¹⁻⁶, which differ in approach and conclusions. We have therefore set out to formulate the problem in a general way in the context of information theory and to draw an analogy with optical data processing systems. There emerge certain general principles which limit the ultimate possibilities of any particular method or algorithm whose purpose is to reconstruct an object from a finite set of its projections. By formulating image reconstruction as an eigenvalue problem we can show how the system is affected by noise. This leads to the idea of a reliable reconstruction, defined in terms of the degree of error which can be tolerated. We then derive expressions showing the dependence of the resolution attainable on the size of the object and the number of projections available.

In considering the resolution achieved, we avoid the use of resolving power, which is not a well defined physical quantity except in the case of an object consisting of two points⁷. We use instead either the radial cut-off R_0 in the spatial frequency spectrum of the object or alternatively the notion of a minimum sampling distance within the object for which density values at neighbouring sample points are independent. If the spectrum of a function contains no frequencies higher than R_0 , the Shannon sampling theorem states that the function is completely specified by its values at a series of points spaced $1/2R_0$ apart. The number P of parameters needed to specify an object to some frequency cut-off may be regarded either as the total number of spatial frequency components or as the total number of independent sample points. For a one-dimensional (real) object of extent $2a$ with a spatial frequency cut-off R_0 , the Shannon sampling theorem gives the total number of independent real parameters or degrees of freedom as $P = 4aR_0$. (The value would be twice as large for an object with a complex density function.) More generally it can be shown⁸⁻¹⁰ that when P is large, it is given in two-dimensional or three-dimensional problems by the product of the respective areas or volumes of the object and of the spatial frequency domain.

In this context the problem of reconstruction may be posed as one of finding out how many degrees of freedom, P' , are determinable from the given set of views. The set of projections acts as a communication channel through which the components

of the object are transmitted with varying strengths. As we shall see, recovery of those components which are transmitted only weakly will be limited by noise. We consider a reconstruction scheme to be reliable only if it is stable against propagation of errors, and a particular reconstructed density to be reliable only if the errors present in the data have not been seriously amplified. We take P' to be the maximum number of parameters that can be extracted from the given projections according to some criterion for the acceptable level of error. P' depends on the number and relative geometry of the various views and also on the maximum spatial frequency present in the data.

In general the P' recoverable parameters will not represent the object uniformly, since not all the components corresponding to a particular magnitude of spatial frequency will be reliably recovered. Details of a given size may be reconstructed in some directions but not others, according to which views are available. Whether a particular non-uniform reconstruction would be useful in practice depends on the nature of the object, for example, its shape and the directionality of its important features. We therefore define a uniform reconstruction as one in which all spatial frequencies out to some cut-off R_0 in the Fourier spectrum are reliably determined. Such a uniform reconstruction will in general contain fewer than the P' degrees of freedom recoverable from the data.

For some applications it is useful to pose the following alternative question. In electron microscopy, in which structure is preserved only to some limiting spatial frequency, R_0 , say, we may ask how many views, say equally spaced, are needed to achieve a uniform reconstruction to this limiting frequency set by the quality of the data. The arguments to be given here need only be rephrased to answer the latter question, however, and we shall not therefore consider it further.

Fourier Analysis

We believe that the Fourier transform provides the most natural and powerful way of analysing the problem of reconstruction. This is not to say that the Fourier transform must be used explicitly in the reconstruction process, but that considering the problem in these terms displays its underlying structure in a rather simple way. The power of the Fourier approach lies in the existence of the projection theorem. This states that the two-dimensional Fourier transform of a two-dimensional projection gives a central section through the three-dimensional transform of the three-dimensional density. Thus sums of numbers in density space, that is projections, are converted into single numbers, that is samples, in the Fourier transform.

The set of such samples given by the available projections does not uniquely determine the whole of the Fourier transform but for a spatially bounded object it will do so out to some

limiting spatial frequency, because the rate of variation of the transform is limited. The object density can then be uniformly reconstructed to the same spatial frequency by the Fourier inversion formula. It is not sufficient to invert the transform using simply the irregularly spaced samples provided by the available projections, because this would lead to a reconstructed density which is distorted by convolution with the transform of the sampling set. The interpolation procedures we have developed² are intended to produce from the irregular samples a regular representation of the transform suitable for inversion.

To bring out the principles we choose the simplest possible situation in which the projection data have been collected by a series of tilts about a single rotation axis. The three-dimensional problem then reduces to a set of two dimensional ones, and the density in each plane normal to the tilt axis is to be determined from a series of line projections. By Fourier transforming each of these line projections we produce a "star" of lines of data in the two-dimensional Fourier transform of each planar density distribution. The continuous transform may be filled in uniquely out to some limiting spatial frequency by a functional expansion over the "star" of sample lines. Beyond this limit the continuous transform is not uniquely determined by the data, and if we attempted a reconstruction to a higher frequency the result would be at best non-uniform and at worst unreliable.

Reconstruction as an Eigenvalue Problem

We now consider the set of functions (objects) that can be reconstructed in an undistorted fashion. We take a function ψ and denote its Fourier transform by $\Psi = F\psi$. If we knew the values Ψ of the continuous transform of ψ , we could immediately regenerate ψ by Fourier inversion, namely $\psi = F^{-1}\Psi$. A finite set of projections of the object specifies the Fourier transform $F\psi$ only on a discrete set or "star" of sampling lines. We denote this sampling operation by S , and the data by $\Psi' = SF\psi$. From Ψ' , we can still formally regenerate ψ by the relation

$$\psi = F^{-1}S^{-1}\Psi'$$

provided that we can produce some operation S^{-1} equivalent to inversion of the sampling operation. The operation S^{-1} must be realized as an interpolation which attempts to span the gaps in the sampled transform and this can only work in a unique fashion if the gaps are not too large. We denote by I the best approximation to S^{-1} that we can produce by interpolation.

The total process of projection and reconstruction then becomes $F^{-1}ISF$ and we would like the function ψ to emerge unaltered from this operation. The best that can generally be done, however, is to regenerate ψ with an altered weight $\lambda \neq 1$. Usually there will be a set of such ψ , which we call ψ_p , each with its own weight λ_p . These ψ_p constitute the eigenfunctions of the total process and represent solutions of the equation

$$F^{-1}ISF\psi_p = \lambda_p\psi_p \quad (1)$$

This can be written in the alternative Fourier form

$$IS\Psi_p = \lambda_p\Psi_p \quad (2)$$

The eigenfunctions ψ_p and eigenvalues λ_p depend on the geometry of the particular set of views that are available but not on the density distribution in the object. They can be determined for various kinds of expansion functions or interpolation schemes, and the results obtained are equivalent to those given by the "least-squares" solution of the interpolation equations described elsewhere².

If we are now given a set of projections of an object, we can consider its density function ρ_0 to be expanded in terms of the appropriate eigenfunctions ψ_p

$$\rho_0 = \sum a_p \psi_p \quad (3)$$

To each ψ_p there corresponds one degree of freedom and the reconstruction process consists in determining as many of the unknown coefficients a_p as the limited data permit. We may expand the transformed projection data Ψ' in the form

$$\Psi' = \sum c_p \Psi_p \quad (4)$$

Fourier transformation of this would produce an image

$$\rho_1 = \sum c_p \Psi_p \quad (5)$$

The relationship of this image to the original object is found by considering formally the result of the total process, namely

$$\rho_1 = F^{-1}ISF\rho_0 = F^{-1}ISF(\sum a_p \Psi_p) = \sum a_p \lambda_p \Psi_p \quad (6)$$

We may identify c_p with $a_p \lambda_p$ and can thus restore the original object by means of the equation

$$\rho_r = \sum \left(\frac{c_p}{\lambda_p} \right) \Psi_p \quad (7)$$

This is the formal solution of the complete reconstruction problem.

Practical Limitations

The form of ρ_r in equation (7) emphasizes that recovery from the data of a given ψ_p present in the original object depends on performing a division (either explicitly or implicitly) by the eigenvalues λ_p . If $\lambda_p = 0$ the corresponding Ψ_p vanishes on all the available sample lines and this implies that the function ψ_p is not represented in the data and cannot be restored. If λ_p is small, the corresponding ψ_p is only weakly represented in the data. Although, with perfect data, the restoration could be performed however small λ_p , provided it is not zero, in practice the restoration of such a ψ_p will be limited by noise. The quantities c_p in the restoration equation (7) are not mere numbers but are the results of measurements and contain errors, ϵ_p say. The restored object ρ_r will therefore contain, besides the desired components $a_p \psi_p$, the noise components $(\epsilon_p/\lambda_p)\psi_p$, because any component $\epsilon_p \psi_p$ will be amplified by $1/\lambda_p$ during the restoration. Thus restoration of eigenfunctions with very small λ_p may so amplify noise present in the data that it completely spoils the restored object density.

The above approach was stimulated by the theory of "super-resolution" in optics^{11,12} where it is mathematically possible to exceed the classical limit of resolution set by the aperture of the lens. This depends, however, on the restoration of eigenfunctions with small eigenvalue¹³ which is not practicable because of noise limitations^{14,15}, unless one is dealing with special classes of object⁷.

For a given noise level in the data we must choose some criterion to judge whether a reconstruction is reliable. The eigenvalues λ_p tend to remain fairly constant with increasing p until some point at which they fall rather sharply. Thus eigenfunctions tend to be either "good" or "bad" and the precise form of criterion is not critical. A possible criterion² might be that the average value of $1/\lambda_p$ should not be greater than unity, so that on average the noise is not amplified during restoration. The number P' of functions satisfying the criterion represents the maximum number of reliably recoverable degrees of freedom contained in the data. Any components not satisfying the criterion should be omitted. A less drastic alternative to complete omission is to restore the weights using a modified formula of the type $c_p/(\lambda_p + \alpha)$ where α depends on the signal to noise ratio. This formula is a special case of Wiener filtering and holds when signal and noise are statistically independent^{14,16}. It has the effect of allowing well determined components to contribute with full weight, while poorly determined ones are included with arbitrary but small weights.

These considerations of noise amplification are of an entirely practical nature but are particularly pertinent in

biological systems, images of which are generally of poor quality. Whatever approach is adopted, it is clear that there is a practical limit P' to the number of terms ψ_p which can be retained and therefore to the resolution which can be achieved. The information about the object that is carried by these components can be found in the data, while all the rest is in practice lost, leading to a reconstruction

$$\rho_r = \sum_p^{P'} \left(\frac{c_p}{\lambda_p} \right) \psi_p \quad (8)$$

Inclusion of all the P' recoverable components will in general give a non-uniform reconstruction. However, they will contain a subset which includes all frequencies out to some cut-off R_0 , and inclusion of just this subset of the recoverable components will lead to a uniform reconstruction.

An Optical Analogy

It is useful to make an optical analogy (Fig. 1) with the reconstruction process considered as two Fourier transformations in sequence. We take an object ψ_p and form with lens L1 its Fourier transform $F\psi_p$ which we sample with a star aperture S . This operation in Fourier space corresponds to forming a set of projections of the original object. If the distribution of light $SF\psi_p$ passes without further modification through the second lens L2 which performs the Fourier inversion F^{-1} , then the image formed will not be a true representation of the object, but will be the latter convoluted with the transform of the sampling function, as would happen if the object were reconstructed directly by "back-projecting" from the projections². In the optical analogy there is relatively too much light passing through the central region of aperture compared with the outer parts. To obtain the correct image we must perform an interpolation operation I to compensate for the missing part of the transform in the blacked-out regions. In practice this might be realized by a density filter (an "inverse filter") whose transmittance increases linearly with radius. The rays $ISF\psi_p$ passing through this compensating filter are combined by lens L2 to form an image $\lambda_p\psi_p$ of the original object, which is undistorted but reduced in amplitude by a factor λ_p .

This optical analogy is not academic. In synthetic aperture optics in which the aperture of the lens is not uniformly filled, a similar deconvolution is required to produce an undistorted image¹⁷. Similar problems arise in radio astronomy^{18,19}.

Analysis in Cylindrical Coordinates

We now examine the behaviour of a concrete example in detail. The results we produce are essentially physical ones and are not strongly dependent on the particular functional expansions used. We assume that we have m projections of a spatially limited object, perpendicular to a single axis, and use cylindrical coordinates (r, ϕ) in the object and (R, Φ) in Fourier space.

An appropriate set of elementary functions in which to expand an object of radius a for this geometry of data is

$$\psi_{ns} = \begin{cases} J_n(2\pi R_{ns}r) \exp(in\phi), & r \leq a \\ 0, & r \geq a \end{cases}$$

where R_{ns} is the s th root of the equation

$$J_n(2\pi R_{ns}a) = 0$$

The Fourier transform of the function ψ_{ns} is of the form

$$\Psi_{ns} = C_{ns}(R) \exp(in\Phi)$$

where $C_{ns}(R)$ has the special property¹⁰ that $C_{ns}(R_{nt})$ is zero if $s \neq t$ but is non-zero for $s = t$. We may thus determine the coefficient of the Ψ_{ns} component by performing an n -fold

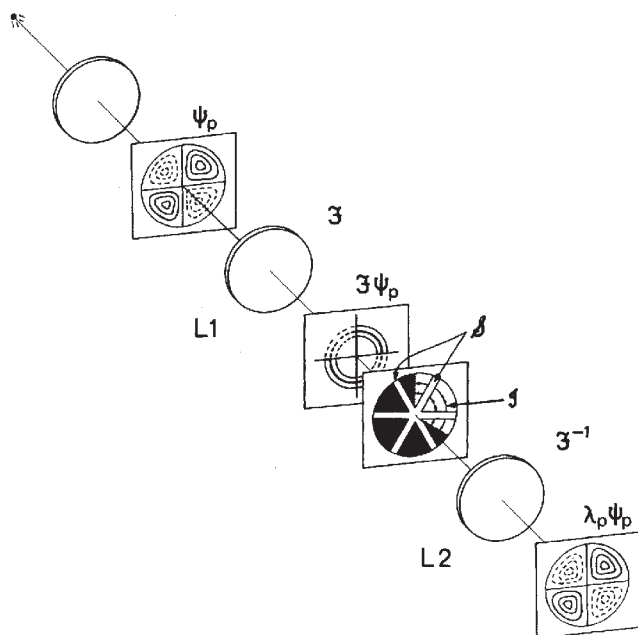


Fig. 1 Transmission of a function ψ_p through an optical system containing a star shaped aperture S in the Fourier plane. For convenience, the masking aperture is drawn displaced from the actual plane. The presence of this mask is equivalent to knowing only the projections of ψ_p on to a set of lines parallel to the lines of the star. The operation I which attempts to restore the lost Fourier components by interpolation is represented schematically as a filling-in of the masked sectors in Fourier space. When this is feasible, the original function is transmitted, but generally with reduced weight λ_p .

harmonic analysis at a radius R_{ns} in the transform. But to convert this to the coefficient of ψ_{ns} in the original object we must divide by $C_{ns}(R_{ns})$, which is therefore equivalent to an eigenvalue for recovery of the radial part of the expansion function ψ_{ns} . If the functions ψ_{ns} are normalized to unity, that is

$$\int_{r \leq a} \psi_{ns} \psi_{ns}^* r dr d\phi = 1$$

so that each degree of freedom is equally weighted in the object, the value of $C_{ns}(R_{ns})$ is given by

$$C_{ns}(R_{ns}) = \sqrt{\pi} J'_n(R_{ns}) \sim (n+2s)^{-1/2} \quad (9)$$

We now consider the azimuthal part of the expansion functions and take first the simplest case when the m projections are equally spaced. From the $2m$ samples available only those Ψ_{ns} with $n \leq n_{max} = m-1$ can be determined. For any particular value of R , the azimuthal variation in the transform is represented by a one-dimensional Fourier series of the form $\sum b_n e^{in\Phi}$ and, if this is known only at $2m$ points, then no harmonics higher than the $(m-1)$ th order can be determined. The highest determinable Ψ_{ns} is therefore $\Psi_{m-1,1}$ and this has its maximum value in the transform at a value of R given by

$$2\pi a R_{m-1,1} \doteq m+3$$

The upper limit R_1 to which the continuous transform of an unknown object can be uniquely determined from m equally spaced views is therefore given by

$$R_1 \doteq \frac{m+3}{2\pi a} \doteq \frac{m}{2\pi a}, \text{ when } m \text{ is large} \quad (10)$$

R_1 represents the maximum cut-off for a uniform reconstruction². Up to this point the azimuthal parts of all eigenfunctions have eigenvalue $2m$, while beyond this point some

eigenfunctions have zero eigenvalues and are not recoverable from the m given views. It is clear that the radial limitation derives from the lack of information in the azimuthal direction.

The eigenvalues given refer to the degree of recoverability of the azimuthal and radial parts separately and the eigenvalues for the whole process will be products of those for the two parts. This separation is convenient for practical computational purposes because only one-dimensional interpolation in angle is being performed. It is possible in principle to exceed the limit $n \leq n_{\max}$ by carrying out two-dimensional interpolation with the complete basis functions. The eigenfunctions are now linear combinations of the ψ_{ns} , and the eigenvalue spectrum does not go exactly to zero at the point $n = n_{\max}$. But it falls sharply just beyond this point so that the extra eigenfunctions have very small eigenvalues and may not in practice be recoverable. It is a moot point whether the few extra degrees of freedom extractable are worth the extra complication of the computation.

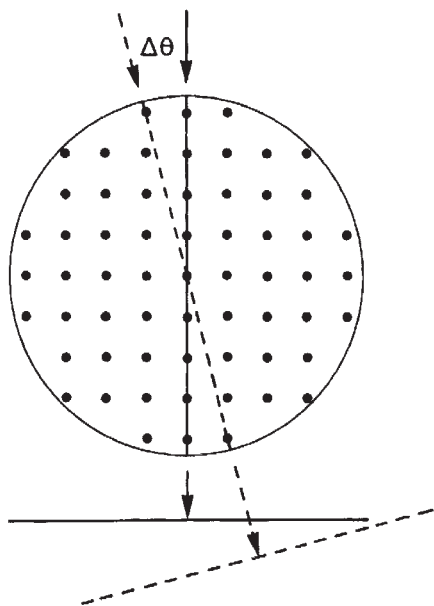


Fig. 2 Two directions of projection showing the minimum angular separation $\Delta\theta$ required in order that different pairs of grid points at the outer edges of the object be superimposed.

The condition (10) can be deduced by a simple physical argument about the filling of Fourier space (see Fig. 1 of ref. 20). We may also interpret this result directly in terms of the independence of different views of the object. If we start with a view which is parallel to the rows of a sampling lattice of spacing $1/2R_1$ (Fig. 2), we must rotate the object by an angle $\Delta\theta$ before some essentially different overlap of lattice points on opposite edges of the object occurs and so gives a distinct view. The angle $\Delta\theta$ is given by

$$\Delta\theta \neq 1/2\pi R_1 \quad (11)$$

Thus in the range $0 \leq \theta < \pi$ there are $2\pi a R_1$ different views of the object, when we consider only spatial frequencies out to a limit R_1 . This is the same number of views as that given by (10) for collecting enough data for an unambiguous, uniform reconstruction right to the edge of the object.

Non-uniform Resolution

The limit R_1 derived above defines a region of Fourier space in which all expansion functions ψ_{ns} can be determined and the transform uniquely specified. The criterion (10) is the most stringent that can be adopted and all points within the reconstruction of a finite body will be equally sharply represented.

The point spread function for such a reconstruction will be of the standard Airy form $2J_1(2\pi R_1 a)/2\pi R_1 a$ and will be independent of the position (r, ϕ) within the object.

It is possible to determine some of the functions ψ_{ns} beyond the cut-off R_1 but the continuous transform is no longer uniquely determined since other components contributing in this region are not recoverable. In the case of equally spaced views it is simple to specify which functions are recoverable. Because we are performing a discrete Fourier inversion in azimuth, "aliasing"²¹ will occur for azimuthal frequencies equal to or greater than m . That is, the weight of any azimuthal component $(m+q)$ present at some radius in the transform will appear added to the weight of the component $(m-q)$ and so prevent the reliable estimation of the $(m-q)$ term. With increasing radius in the transform more and more components are dropped (Fig. 3) until at a radius of

$$R_3 \frac{1}{2} \frac{2m+4}{2\pi a} \frac{m}{\pi a} \quad (12)$$

the J_{2m} contribution becomes significant so that finally even the J_0 contribution becomes irrecoverable. Inclusion of all the functions ψ_{ns} not affected by aliasing will, however, lead to a very non-uniform reconstruction, in which features at the centre of the object are represented to approximately twice the spatial frequency and with about twice the peak height as those at the edge. This effect has been discussed by Gilbert^{22,23}.

The $(n+2s)$ Cut-off

There is a good case for including some of the functions beyond the R_1 cut-off which are not affected by aliasing. All functions with a given value of $(n+2s)$ have the same degree of recoverability of their radial parts (9) above) and thus their inclusion leads to the same level of noise amplification.

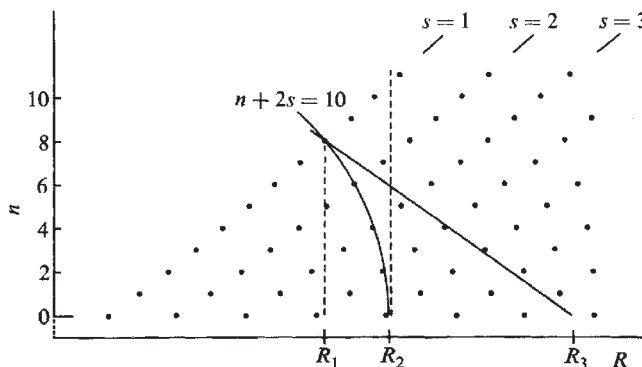


Fig. 3 Plot showing the relative positions, indicated by the dots, of the various roots j_{ns} of $J_n(x)=0$ for each Bessel order n . We may interpret the horizontal scale as a reciprocal radial coordinate R , which takes the particular values $R_{ns}=j_{ns}/2\pi a$ when the functions $J_n(2\pi R_{ns}r) \exp(in\phi)$ are used to expand an object of radius a . The Fourier transform $C_{ns}(R) \exp(in\phi)$ of each of these functions is concentrated mainly at a radius R_{ns} . Various cut-offs appropriate to nine equally spaced views are shown. The highest azimuthal order determinable without aliasing is $n=8$ and R_1 gives the corresponding radial cut-off out to which all functions contributing to the transform can be determined. The $(n+2s)$ cut-off for this case is $(n+2s) \leq 10$, for which the extreme function is C_{05} at a radius R_2 . The $(n+2s)$ cut-off is slightly non-uniform, because the functions $C_{91} C_{10,1} C_{82}$ which would come within a cut-off at R_2 must be excluded to avoid aliasing. The aliasing boundary is indicated by the line sloping to R_3 ; functions below this line are determinable while those above are not.

Another basis for this choice is that a one-dimensional projection of the function ψ_{ns} contains $(n+2s)$ zeroes within the limits of the projection. The zeroes are approximately equally spaced and so all functions corresponding to a given value of $(n+2s)=n'$ can be considered to represent the projec-

tions with the same resolution distance equal to the diameter $2a$ of the object divided by $n'/2$, or $4a/n'$. A measure R_2 of the effective resolution achieved in the reconstruction is the reciprocal of this distance for the highest recoverable harmonic n_{max} , namely

$$R_2 \doteq \frac{n_{max}}{4a} \doteq \frac{m}{4a} \quad (13)$$

This value is also given by considering the position of the peak of $C_{0,m,2}$ (Fig. 3).

We obtain an identical result when the analysis is carried out using "circular" prolate spheroidal functions²⁴, because the eigenvalue spectrum for these functions also appears to depend on the value of $(n+2s)$ (Fig. 2 of ref. 23). Taking the point $\lambda=1/2$ as the cut-off in the spectrum gives the expression (13) for R_2 . The majority of the functions Ψ_{ns} contributing to the transform between the limits $m/2\pi a$ and $m/4a$ are included on the $(n+2s)$ criterion (Fig. 3). The resulting reconstruction should thus be almost uniform and this has been confirmed by computing the point spread function at different positions in the object²⁵. The relatively few functions omitted carry information about the outermost parts of the object.

The number of expansion functions satisfying the condition $n+2s \leq n_{max}+2$ is approximately $\frac{1}{2}n_{max}^2$. Since the functions are complex, the number of real degrees of freedom determin-

able from m views and leading to a reasonably uniform reconstruction is therefore

$$P \doteq \frac{1}{2}n_{max}^2 \doteq \frac{1}{2}m^2 \quad (14)$$

This result, which is to be understood in the asymptotic sense for large m , can be used to give another estimate of the resolution R_2 by invoking the Gabor expansion theorem to equate $\pi a^2 \pi R^2$ to $\frac{1}{2}m^2$, yielding

$$R_2 \doteq \frac{m}{\sqrt{2\pi}a} \doteq \frac{m}{4.5a} \quad (13')$$

The maximum number of independent samples of the density in the original object that can be obtained from m projections is also given by $\frac{1}{2}m^2$. The area (volume) of the sample cells is proportional to the area (volume) of the object. In any reconstruction method which uses, or purports to yield, a finer uniform subdivision of the original object, the densities at neighbouring grid points will not be independent, particularly at the outside of the object.

Restricted Range of Views

If the m views are not equally spaced, it is not the basis functions Ψ_{ns} themselves which are eigenfunctions but rather linear combinations of them. The corresponding eigenvalues λ_{ns} obtained from one-dimensional interpolation in azimuth lie between $2m$ and 0 but fall to zero sooner than in the equally

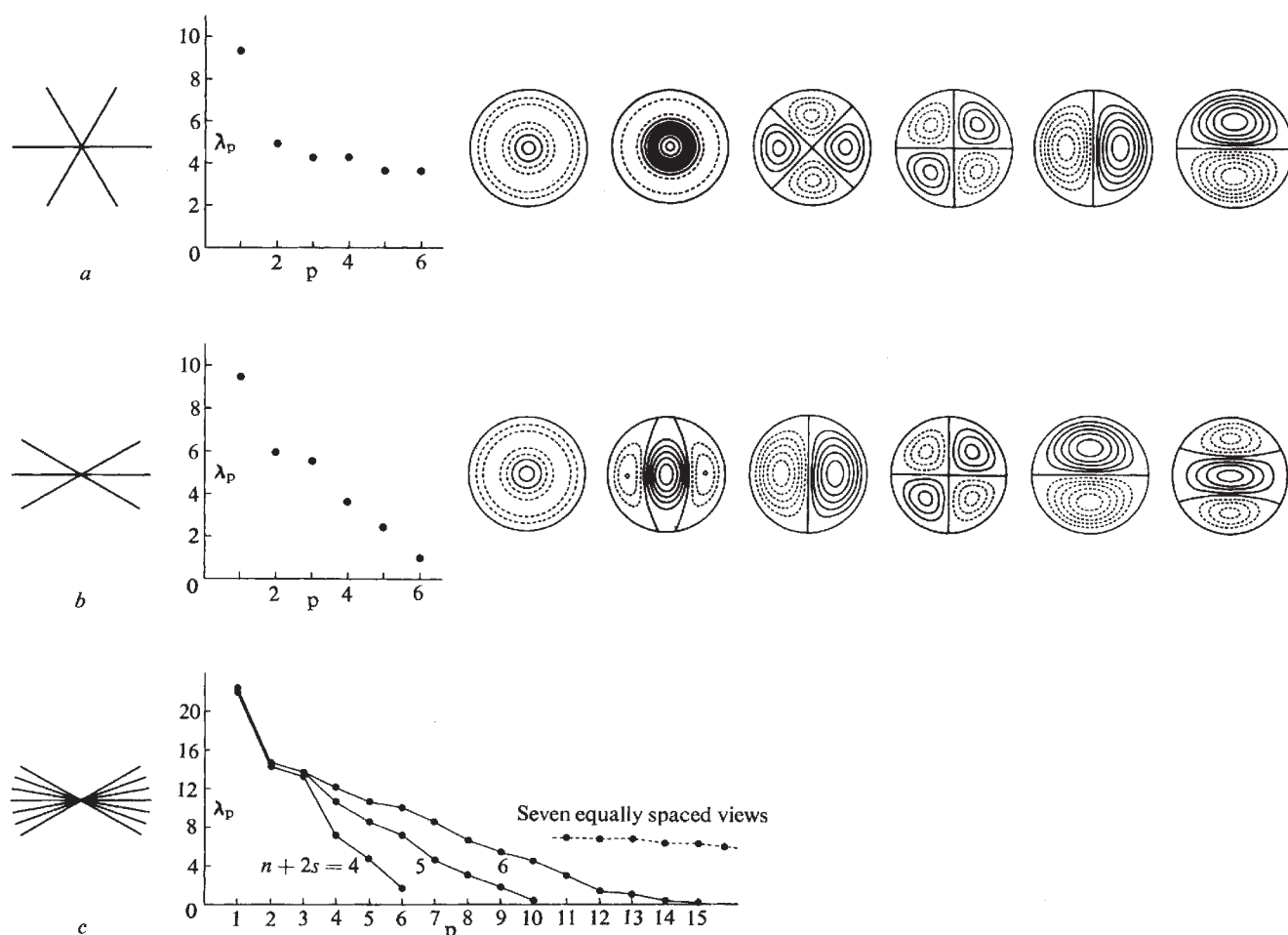


Fig. 4 Eigenvalue spectra λ_p and eigenfunctions, in decreasing order of eigenvalue, for (a) three equally spaced projections and (b) three projections in a range of 60° . The eigenfunctions are constructed out of six basis functions, namely ψ_{01} , ψ_{02} and the real and imaginary parts of ψ_{11} and ψ_{21} , corresponding to the uniform cut-off $(n+2s) \leq 4$. They were computed by two-dimensional interpolation and are linear combinations of the Ψ_{ns} . In (b) the eigenfunctions with the smallest eigenvalues carry information about the object in the "vertical" direction for which no projection is available. (c) Eigenvalue spectra for seven views restricted to a range of 60° , corresponding to an $(n+2s)$ cut-off respectively of 4, 5 and 6. For $(n+2s) \leq 7$ some eigenfunctions have an eigenvalue so small ($\lesssim 10^{-2}$) as to be in practice irrecoverable. The effect of dropping these eigenfunctions would be to give a non-uniform reconstruction. The maximum uniform cut-off in this case would appear to be $(n+2s) \lesssim 6$. This should be contrasted with the case of seven equally spaced views for which the cut-off $(n+2s) \lesssim 8$ applies, giving a spectrum shown (in part) by the dotted line.

spaced case. If the m views are clustered there will be correspondingly large gaps in our knowledge of the Fourier transform. This then implies the existence of eigenfunctions whose major contributions in Fourier space lie within these gaps and which therefore have small imaging weights λ_{ns} over the restricted range of projections.

Thus in the example of Fig. 4 restriction of the three views to a range of 60° has the effect of reducing the smallest eigenvalue by a factor of about 4. When the projections are clustered normal to a preferred direction (Fig. 4b) the poorly determined eigenfunctions are those which represent variations in density parallel to this preferred direction so that the resolution is poor in this direction. This situation would obtain in electron microscopy if only a limited range of tilts about a single axis is available. The lack of information when the views are clustered cannot be made good simply by including more views within the same restricted range. Thus, for the same number of basis functions, increasing from three to seven the number of views within the restricted range of 60° (Fig. 4c) does not improve the ratio of maximum to minimum eigenvalue. Even if the restricted range were filled continuously with projections there would still be low order eigenfunctions with small λ and therefore not recoverable (compare ref. 26). In an alternative scheme of reconstruction, where the projection equations are solved directly without using Fourier transforms², increasing the number of views without at the same time increasing the range also shows a similar lack of improvement in the conditioning of the equations.

The set of spectra in Fig. 4c also illustrates the point that when the views are very unequally spaced, fewer eigenfunctions, and therefore fewer basis functions, may be reliably determined than in the case of equally spaced views. Thus, for a prescribed value of λ and therefore of noise amplification, a uniform reconstruction can be made only to a lower spatial frequency (see legend).

Concluding Remarks

The implementation of different reconstruction schemes and their practical application to electron microscopy have already been described elsewhere²⁷⁻³⁰. The problem of image reconstruction also arises in X-radiography and solutions have been proposed by others^{31,32}. The Fourier approach that we have adopted brings out the similarity between image reconstruction and certain techniques in the radio astronomy^{18,19}. We have here cast the problem in a general form which brings out the limitations imposed by having only restricted data available.

Although one reconstruction scheme may be preferable to another in computational terms, claims that a particular reconstruction scheme for a general object can in principle operate with fewer data than another should be viewed with some scepticism. We³³ have already discussed the "ART" method⁵. The apparent success of the examples given is due to the fact that a special procedure was used in generating the data used in the model reconstructions, and indeed the ART algorithm is unstable against propagation of errors³⁴. A different iterative method (SIRT) has been proposed³⁴ which produces results in close agreement with Fourier methods. A recent statement⁶ that "it appears possible to obtain reasonably good reproduction with only a small number (about 3-12) of transmission photographs" is not accompanied by any analysis of the resolution achieved, and, in the model examples shown, only low spatial frequencies are recovered. In view of the equivalence of the convolution method used⁶ and the Fourier method, it is difficult to see how the former could be any more powerful.

The method of "projection functions"⁴ which is a form of "back-projection"² produces a reconstruction in which the true density is convoluted with the transform of the sampling function. This will give a reasonable reconstruction when the object consists of strong peaks of density. We do not agree, however, with the statement⁴ that the "resolution" achieved

is, in our notation, $R = m/2a$ because this would only be true if all density samples in all projections were independent. While the density samples in any one projection are independent, the accumulated samples in a set of projections are not. As can be seen from the Fourier transform approach the low spatial frequencies are correlated, being determined repeatedly by the various projections, while the high frequencies remain relatively independent. A derivation which allows for this effect leads to our criterion (10) for an unambiguous reconstruction.

In summary we have analysed the problem of three-dimensional reconstruction of a general object in terms of the number of degrees of freedom reliably recoverable from a limited set of projections. The analysis takes a simple form when use is made of Fourier transforms, but the conclusions are completely general. By considering reconstruction as an eigenvalue problem we have dealt with the crucial problem of the amplification of noise, which sets fundamental limits on what can be achieved in practice by any scheme of reconstruction. A reliable reconstruction scheme is one which extracts maximum information from the data while preventing the spoiling of the results by effects which are beyond resolution with the limited number of views available. Criteria have been given for reconstructing general objects about which no *a priori* information is available. If, however, the object is known to consist, for example, of point-like features the whole situation is quite different. Similarly if the reconstruction has a rather limited objective, such as locating a strong feature in a known class of object—a situation which may arise in radiography—less stringent criteria can be adopted. But, if the object is general and the projections contain noise, no mathematical or computational manipulation can make up for a lack of information in the data.

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Tidal Resonance in the Bay of Fundy and Gulf of Maine

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Tidal ratios outside and inside the Bay of Fundy/Gulf of Maine system fall into two distinct groups. The period of the free mode oscillation in the bay is 13.3 h, and this is forced by the lunar M_2 tide of the North Atlantic to produce the unusually high tides recorded in the area.

THE tides in the Bay of Fundy are probably the highest in the world, with a range occasionally greater than 50 foot at places in Minas Basin at the head of the bay. The tides are dominated by the principal lunar semi-diurnal constituent M_2 , with a period of 12.42 h, although S_2 (the principal solar semi-diurnal constituent, with period 12 h), N_2 (the larger lunar elliptic constituent, arising from the fluctuating distance of the Moon from Earth, with period 12.66 h) and the diurnal tides also make significant contributions.

Honda *et al.*¹ and Proudman² attributed the large range to quarter-wavelength resonance near the M_2 period, but Redfield³ and Ippen and Harleman⁴ disputed this and Rao⁵ calculated the period of the lowest mode in the Bay of Fundy to be 9.047 h. Duff⁶ argued that part of the Gulf of Maine must also be taken into account, and that it is this larger system which is close to resonance, but he did not estimate the resonant period or Q .

The obvious way to determine the resonant frequency of a system is to examine its response for a range of frequencies, but in spite of nature's comparative generosity in forcing the oceans at several different frequencies, this method has apparently not been applied to the Bay of Fundy. Harmonic constants (amplitudes and phase lags behind the astronomical forcing functions) for M_2 , S_2 , N_2 are generally determined accurately from records of sea level over a year or more. The smaller semi-diurnal constituents are either so close in frequency to the principal constituents that they do not provide significant extra information, or submerged in the non-tidal noise⁷, or generated by non-linear interaction of the principal constituents as well as by linear response to astronomical forcing. In this investigation of a possible resonance with a nearly semi-diurnal frequency I shall thus confine my attention to M_2 , S_2 and N_2 with frequencies $\omega_M = 1.932$ cycles per day (c.p.d.), $\omega_S = 2.000$ c.p.d. and $\omega_N = 1.896$ c.p.d., respectively.

Tidal Ratios

Table 1 shows the tidal ratios (amplitude ratios and differences in phase lag behind the forcing function) for M_2 , S_2 , N_2 for ports on the eastern seaboard of the United States and Canada, outside and inside the Bay of Fundy and Gulf of Maine. The locations of ports used are shown in Fig. 1. Apart from Sable Island, only ports for which the harmonic constants have been derived from sea level records covering at least a year are listed. The ports of Table 1a are further restricted to be fairly close to the Gulf of Maine, and ports in or near Long Island

Table 1 Tidal Ratios Outside and Inside the Bay of Fundy/Gulf of Maine System

Port	$g(S_2)/g(N_2)$	$g(S_2)/g(M_2)$	$H(N_2)/H(S_2)$	$H(M_2)/H(S_2)$
(a) Outside				
Sable Island	50.0	33.0	0.789	4.526
Halifax	46.4	28.0	0.998	4.400
New York (Battery)	44.5	25.7	1.073	4.816
Sandy Hook	41.9	26.9	1.056	4.800
Bermuda *	40	22	1.01	4.43
(b) Inside				
Yarmouth	58.4	30.0	1.299	6.077
Digby	61.7	36.4	1.233	6.231
Saint John	61.7	35.7	1.310	6.113
Eastport	69.2	38.2	1.248	6.008
Portland	64.9	34.4	1.366	6.135
Boston	64.3	35.2	1.377	6.145

* Based on average harmonic constants for St George's Island and Great Sound.

H is the height of the constituent and g its phase lag in degrees behind the corresponding astronomical forcing function.

Sound (which has response characteristics of its own) have been excluded.

Although there is some scatter, it is clear that the tidal ratios outside and inside the Bay of Fundy/Gulf of Maine system do fall into two distinct groups. I assume that the tidal ratios in Table 1a are representative of the regional relationships for the western North Atlantic near the Gulf of Maine. The tidal ratios for Bermuda (1,000 km south of the Gulf of Maine) included in Table 1 are not greatly different from the tidal ratios for the other ports and so support this assumption. Sable Island is perhaps best placed for measurement of the appro-

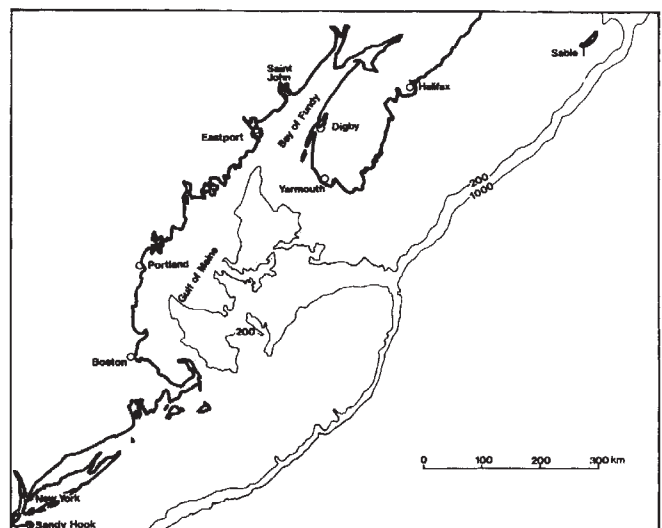


Fig. 1 The Bay of Fundy and Gulf of Maine. Tidal ratios are used for the ports marked. The 200 m and 1,000 m bathymetric contours are shown.

appropriate values, but the erratic agreement between the tidal ratios for Halifax and Sable Island suggests that the two 29-day records and one 15-day record from Sable Island used for determination of the harmonic constants were too short for the values to be trustworthy.

The consistency of the tidal ratios for the ports of Table 1b suggests that, contrary to Duff⁶, Boston, and indeed the whole Gulf of Maine, is part of a single system with the Bay of Fundy.

Table 2 Average Values of the Tidal Ratios Outside and Inside the System

	$g(S_2)-g(N_2)$	$g(S_2)-g(M_2)$	$H(N_2)/H(S_2)$	$H(M_2)/H(S_2)$
Outside	44.2 ± 2.2	26.9 ± 1.1	1.036 ± 0.037	4.61 ± 0.21
Inside	63.4 ± 2.2	35.0 ± 1.7	1.306 ± 0.035	6.12 ± 0.045

Average values for the tidal ratios are given in Table 2.

The range of values outside includes the values for Halifax, New York and Sandy Hook. Inside there are rather more data, so I have taken the mean $\pm 1.5 \times$ the standard error of the mean (for about 90% probability of the correct values) for the 6 ports. I define

$$\begin{aligned}\theta &= [g(S_2)-g(N_2)]_{\text{inside}} - [g(S_2)-g(N_2)]_{\text{outside}} = 19.2 \pm 4.4 \\ \varphi &= [g(S_2)-g(M_2)]_{\text{inside}} - [g(S_2)-g(M_2)]_{\text{outside}} = 8.1 \pm 2.8 \\ \lambda &= [H(N_2)/H(S_2)]_{\text{inside}} \div [H(N_2)/H(S_2)]_{\text{outside}} = 1.26 \pm 0.08 \\ \mu &= [H(M_2)/H(S_2)]_{\text{inside}} \div [H(M_2)/H(S_2)]_{\text{outside}} = 1.33 \pm 0.07\end{aligned}$$

M_2 and N_2 are both amplified more than S_2 , and their phase lags increase less than that of S_2 .

Response Function

The tide in a gulf is forced chiefly by the tide in the open ocean⁸, rather than directly by the Sun and Moon. The M_2 tide in the Bay of Fundy actually does work on the Moon⁹, but only at the rate of about 8% of the total dissipation within the bay. For the moment I shall neglect this small direct (negative) forcing.

I assume that the response of the system is largely in one mode, and that the tidal elevation $\eta(x, t; \omega)$ at position x , time t and frequency ω is

$$\eta(x, t; \omega) = \mathcal{R}_e A(\omega) S(x) \left[\frac{\omega_0 - \omega}{\omega_0} - \frac{1}{2} i Q^{-1} \right]^{-1} e^{-i\omega t} \quad (1)$$

$A(\omega)$ is the (complex) amplitude of a tidal constituent of frequency ω outside the system; more precisely, it is the amplitude at the entrance to the system if the system were closed off. I assume that the shape of this amplitude across the entrance is the same for all semi-diurnal frequencies, as is indicated by the near equality of the tidal ratios at Halifax, New York and Sandy Hook.

$S(x)$ is the (complex) spatial dependence of the single mode. If one mode is close to resonance, it will dominate. Moreover, if more than one mode contributed to the response, the tidal ratios should vary significantly within the system¹⁰, and this appears not to be the case.

The denominator of equation (1) describes a simple resonance at frequency ω_0 with a dissipation of $2 \pi Q^{-1}$ of the energy of each cycle. Theories of harbour resonance^{11,12} indicate that this is an appropriate form for the denominator for excitation of the lowest mode (quarter wavelength or Helmholtz), which is almost certainly present here. For the higher modes the response is best discussed in terms of an equivalent electrical circuit¹², and that part of Q due to radiative damping is dependent on $(\omega - \omega_0)$.

Resonant Frequency and Q

The ratios of $A(\omega)$ for different frequencies are given in amplitude and phase by the tidal ratios outside the system. The differences in tidal ratios inside and outside the system are

thus due to the variation of ω in the denominator of equation (1), and so

$$\lambda e^{-i\theta} = \frac{\left(\frac{\omega_0 - \omega_S}{\omega_0} \right) - \frac{1}{2} i Q^{-1}}{\left(\frac{\omega_0 - \omega_N}{\omega_0} \right) - \frac{1}{2} i Q^{-1}} \quad (2)$$

$$\mu e^{-i\varphi} = \frac{\left(\frac{\omega_0 - \omega_S}{\omega_0} \right) - \frac{1}{2} i Q^{-1}}{\left(\frac{\omega_0 - \omega_M}{\omega_0} \right) - \frac{1}{2} i Q^{-1}} \quad (3)$$

relating the two unknowns ω_0 and Q to the four observed quantities λ , μ , θ , φ . For reasons that will emerge shortly, I determine ω_0 , Q from equation (2) as

$$\omega_0 = \omega_S - (\omega_S - \omega_N) \lambda (\lambda - \cos \theta) (\lambda^2 - 2\lambda \cos \theta + 1)^{-1} \quad (4)$$

$$\frac{1}{2} Q^{-1} \omega_0 = (\omega_S - \omega_N) \lambda \sin \theta (\lambda^2 - 2\lambda \cos \theta + 1)^{-1} \quad (5)$$

For the possible ranges of λ , θ , this gives ω_0 between 1.75 c.p.d. and 1.86 c.p.d. (resonant period between 12.9 and 13.8 h), and Q between 3.1 and 5.6. Substitution of possible solutions of (2) into (3) gives values of μ that are all too small, and values of φ that are generally too large; rather than reject the model completely, I shall introduce more physics.

Frictional Damping for a Mixture of Tides

Q is determined by radiative loss of energy into the Atlantic and by frictional dissipation within the system. The former is a linear process and, as already argued, can reasonably be taken as frequency-independent in this case. The latter process is non-linear, the frictional force opposing a current u generally being taken as proportional to $u|u|$. Jeffreys¹³ showed that for $u = \cos \omega_1 t + \varepsilon \cos \omega_2 t$, the Fourier component of $u|u|$ at frequency ω_2 is $1.5\varepsilon + O(\varepsilon^3)$ times the Fourier component at frequency ω_1 . Thus if a current is composed of one large tidal constituent and a number of much smaller ones, the larger constituent has a dissipative Q 50% greater than that of the smaller constituents. This remarkable result is readily extended to the situation in which the current has components in two directions, but with tidal ellipses of the same eccentricity for all the constituents being considered. A simple interpretation of the result is that quadratic damping will be greater than average for large tides and less than average for small tides. This will reduce any modulation, which is equivalent to greater damping of the smaller constituents.

To allow for this I define Q_{SN} as the total Q for S_2 , N_2 , Q_M as the total Q for M_2 and $c = Q_{SN}/Q_M$. We require $2/3 \leq c \leq 1$, the lower limit corresponding to limitation of the resonance entirely by friction without any radiative loss and the upper limit corresponding to the other extreme. The effect of dissipation on the resonant frequency ω_0 is quadratic in the damping rate⁵, so that in this case the difference in ω_0 for M_2 on the one hand and S_2 , N_2 on the other is probably less than 1% and negligible. The differential dissipation probably also makes $S(x)$ slightly different for M_2 , but the relative constancy of the tidal ratios inside the Bay of Fundy and Gulf of Maine indicates that the effect of this is negligible. Thus I replace equation (3) by

$$\mu e^{-i\varphi} = \frac{\left(\frac{\omega_0 - \omega_S}{\omega_0} \right) - \frac{1}{2} i Q_{SN}^{-1}}{\left(\frac{\omega_0 - \omega_M}{\omega_0} \right) - \frac{1}{2} i c Q_{SN}^{-1}} \quad (6)$$

This may be solved for c , μ , using the values of φ_0 , Q_{SN}

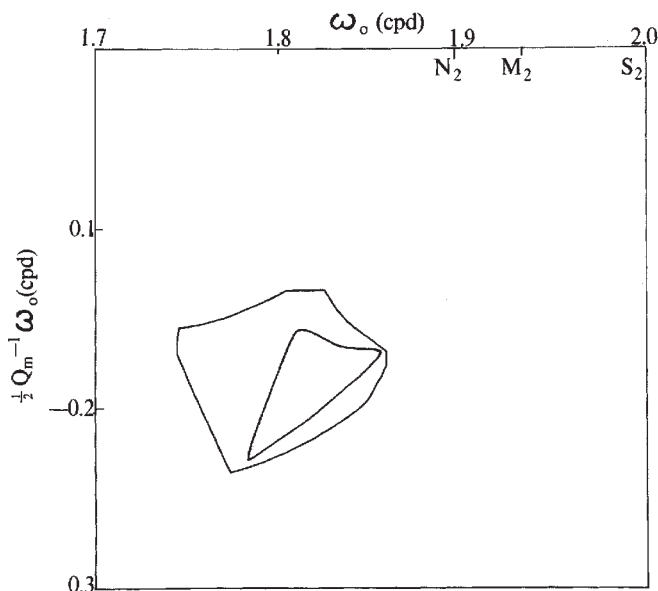


Fig. 2 Subject to the possible ranges of the tidal ratios, the point $(\omega_0 - \frac{1}{2}iQ_M^{-1}\omega_0)$ lies within the enclosed region. If we further require $Q_D < 10$, the point is restricted to the shaded area.

given by equations (4) and (5), and for the observed range of φ :

$$c = \frac{2Q_{SN}(\omega_0 - \omega_M)[\omega_0 - 2Q_{SN}(\omega_0 - \omega_S)\tan\varphi]}{\omega_0[2Q_{SN}(\omega_0 - \omega_S) + \omega_0\tan\varphi]} \quad (7)$$

$$\mu = \left[\frac{4Q_{SN}^2(\omega_0 - \omega_S)^2 + \omega_0^2}{4Q_{SN}^2(\omega_0 - \omega_M)^2 + c^2\omega_0^2} \right]^{\frac{1}{2}} \quad (8)$$

The value of μ thus derived may be compared with the data.

If Q_R denotes the radiative Q (common to all constituents), and Q_D denotes the dissipative Q for M_2 ($= 1.5 \times$ the dissipative Q for S_2, N_2),

$$Q_M^{-1} = Q_R^{-1} + Q_D^{-1}, \quad Q_{SN}^{-1} = Q_R^{-1} + 1.5 Q_D^{-1} \quad (9)$$

$$\text{Hence} \quad Q_R = Q_{SN}/(3c - 2), \quad Q_D = \frac{1}{2}Q_{SN}/(1 - c) \quad (10)$$

Anomalous Q_R/Q_D Values

Using the central values of θ, λ, φ , I obtain

θ (°)	λ	φ (°)	ω_0 (c.p.d.)	Q_{SN}	c
19.2	1.26	8.1	1.80	4.34	0.881
$\mu \quad Q_M \quad Q_R \quad Q_D \quad P_0 (= 24\omega_0^{-1} \text{ h})$					
1.28	4.93	6.77	18.17	13.33	

The calculated μ is lower than the central observed value (1.33), but still within the permissible range. Fig. 2 shows the region of the complex plane in which $\omega_0 - \frac{1}{2}iQ_M^{-1}\omega_0$ may lie for $\theta, \lambda, \varphi, \mu$ within the permitted ranges, and $\frac{2}{3} < c < 1$.

That value of Q_D seems rather high. Redfield's³ damped wave analysis of the M_2 tide in the Bay of Fundy suggests a Q_D of about 3 for this region alone. Many authors have estimated the flux of energy into the Bay of Fundy, with fairly consistent results. The most recent value¹⁴ is 2.8×10^{10} W, of which 2.5×10^{10} W is dissipated if one retains McLellan's⁹ value for the rate of working by the tides on the Moon. Taken with Yuen's¹⁵ estimate of 1.54×10^8 kWh for the maximum potential energy in the Bay of Fundy, and assuming that the average kinetic and potential energies are both half as much, this also gives $Q_D \div 3$. Of course the rest of the Gulf of Maine is far less dissipative, but it also has a much lower energy density. It seems unlikely that Q_D for the whole system could be more than about 10. The shaded area of Fig. 2 indicates the region within which the solution lies if we require $Q_D < 10$.

The large mouth of the Gulf of Maine suggests a very low Q_R , but the southern part of the gulf is more or less cut off from the open ocean by shallow water. The principal entrance to the system is the Fundian Channel (outlined by the 200 m isobath in Fig. 1) which is rather narrow, and Miles¹⁶ has shown that an abrupt change in depth, as at the edge of the continental shelf, can also increase Q_R .

Future Tidal Amplification?

My calculations suggest that the Bay of Fundy and Gulf of Maine have a resonant period of 13.3 ± 0.4 h and that the present M_2 tide has a Q of 5.25 ± 1.5 . Smaller constituents have the same resonant period but a slightly smaller Q .

Perhaps the greatest assumption of this study is neglect of direct forcing by Sun and Moon. As already mentioned, in the Bay of Fundy the M_2 tide does work on the Moon at 8% of the dissipation rate. Owing to the increasing phase lag with frequency the associated damping is less important for S_2 but more important for N_2 . So it is apparent qualitatively that the observed changes in tidal ratios tend to underestimate the amplification of N_2 and M_2 relative to S_2 that would occur in the absence of direct forcing within the system. Any attempt to allow for this quantitatively is bound to be rather approximate, but rough calculations indicate that the resonant period may be somewhat increased to around 14 h and the Q somewhat decreased to 3 or 4.

There can be little doubt that the resonant period is greater than the M_2 period of 12.42 h. Construction of barriers in the upper part of the Bay of Fundy for the generation of electrical power would change the tidal amplitude at any point in the Bay of Fundy by changing $S(x)$, Q_R and Q_D , but, energy extraction apart, chiefly by increasing ω_0 . Unless the change of geometry is drastic enough to put ω_0 further above ω_M than it is at present below it, a slight amplification of the tides appears likely. Much further work is required to predict the changes with any confidence. Previous work on this problem^{6,15,17,18}, which predicted a decrease in tidal amplitude, considered too small a region and was based on the false assumption that the tidal amplitude at the outer boundary of the system would be unchanged by barrier construction, an assumption contrary to results from studies of analogous problems^{11,12}.

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General Theory of Chromosome Structure and Gene Activation in Eukaryotes

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This simple theory provides a function for non-histone proteins and an explanation for the large size of eukaryotic genomes, repetitive sequences in DNA, HnRNA and "processing" of nuclear RNA.

It is generally assumed that, in eukaryotes, gene activity is regulated in the same way as in prokaryotes. Although some evidence suggests that this is the case¹, many of the features of eukaryotic chromosomes seem to be incompatible with prokaryotic models. Both prokaryotic and eukaryotic chromosomes are probably based on a single DNA double helix, but in eukaryotes the total genome may be 100–10,000 times larger than in *E. coli*. A variable amount of the DNA, of the order of 50%, may consist of "unique" sequences, that is, sequences which, as tested by reannealing, are present only once in the genome. If these sequences represent structural genes this implies an information content in a mammalian cell about 500 times greater than in *E. coli*; that is, about one million genes coding for proteins of 20,000 MW. Haldane², Müller³, and Ohno⁴ have pointed out that this number of genes would probably impose an impossible mutational load. We conclude either that the mutational load argument does not hold for eukaryotes, or that much of the DNA in eukaryotes is not informational. The remainder of the DNA in eukaryotes consists of repetitive sequences which occur in families, each containing some tens or hundreds of thousands of non-identical but very similar members⁵; the functions of repetitive classes of DNA are not known. Whereas the bacterial chromosome consists essentially of naked DNA with which small numbers of protein molecules are briefly associated, the prokaryotic chromosome contains at least as much permanently associated protein as DNA. There are two classes of proteins, histones and non-histones. The function of the histones is unknown, but their association with DNA leads to the formation of a compact, possibly supercoiled, structure which is relatively inactive in RNA synthesis⁶. To facilitate transcription, the neutralization of this effect of histones is required, and the evidence suggests that non-histone proteins perform this function⁷.

The giant inter-phase chromosomes of diptera show a characteristic banding pattern; each band plus interband probably corresponds to a complementation group⁸. The band regions contain high concentrations of DNA and histones, and probably consist chiefly of irregularly supercoiled or folded nucleohistone⁹. The less dense interband regions probably consist of less supercoiled nucleoprotein. The band patterns are generally typical for a species; usually there is no tissue specificity in their distribution. Some regions, however, expand to form diffuse structures called "puffs"; their distribution is tissue specific. Besides DNA and histones, the puff regions contain non-histone proteins and RNA, and are

active in RNA synthesis. Non-puffed regions are much less active in RNA synthesis, although it is not certain that they are completely inactive; they contain little, if any, non-histone protein or RNA¹⁰.

The lampbrush chromosomes in oocytes have an analogous structure. The condensed regions, chromomeres, may correspond to bands in dipteran chromosomes. In some regions loops, analogous to puffs, extend from the axis; in these RNA synthesis occurs. The loops extend at the expense of chromomeric material¹¹.

It has been shown, by a variety of staining methods, that mammalian mitotic chromosomes exhibit a banded structure as well. Although the banding so far demonstrated is much coarser than that in giant or lampbrush chromosomes, and may not be related to it, it is species-specific¹².

I believe that eukaryotic chromosomes have a characteristic structure in which tightly compacted nucleoprotein (globular DNA of Crick¹³) alternates with loosely compacted nucleoprotein or fibrous DNA according to a fixed pattern. Although it is not certain where transcription originates in relation to tightly compacted and loosely compacted nucleoprotein, it clearly extends into the compact chromomeric regions and results in the formation of diffuse chromatin as loops or puffs.

Structure of Compact and Diffuse Regions

Histones are, in general, randomly distributed over DNA in chromatin but, as the chromosome is not uniform but has the characteristic banded pattern described above, I conclude that the compact and less compact regions have different chemical structures. Since nucleohistone alone forms very compact masses in ionic conditions similar to those in the nucleus, the implication is that the less compact regions contain modified histones, modified DNA or extra substances which modify the conformation of nucleohistone. Because histones do not endow reconstructed nucleoprotein with transcriptional specificity¹⁴, the first possibility is unlikely. Furthermore, it is now difficult to envisage structures for two different molecules of much the same base composition which, when associated randomly with histones, would form either a tightly coiled structure or a loosely coiled one. For these reasons I suggest that less compact regions contain an extra substance which prevents tight supercoiling. Some direct evidence for this comes from experiments^{10,15} conducted many years ago which showed that the interband regions are specifically sensitive to pepsin treatment. If extra molecules are required in these regions they must bind to specific sites. One is thus led to postulate that each less compact region is also characterized by a special DNA structure.

In nucleohistone the pitch of the helix is somewhat less than in free DNA¹⁶. An effect of histones is, therefore, to tighten the helix, and it may be the application of this force which produces an irregular supercoil of 100–120 Å diameter and supercoiling of higher orders. The presence of polyanions in a complex of DNA, histones and polyanions would almost certainly have an effect of reducing the twisting produced by histones, by competing with DNA for some of the positive

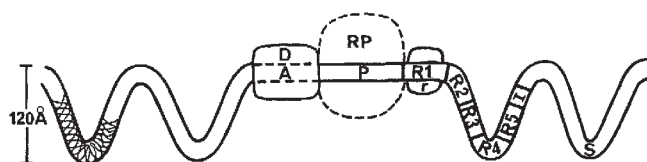


Fig. 1 Postulated structure of a transcribable region of DNA in chromatin. Nucleohistone is assumed to form a supercoiled structure with a diameter of approximately 120 Å. A transcribable region of DNA is postulated to contain an address site (A) closely linked to a promoter site (P), regulator sites (R1, R2, etc.) and an initiator site (I). A destabilizing molecule (D) binds to A to cause local relaxation of supercoiling, which permits a molecule of RNA polymerase (RP) to attach to the promoter. If no repressors (r) are bound to regulator sites transcription is initiated and the structural gene (S) is transcribed.

charges on histones, and by generally increasing the ionization of phosphates in the DNA backbone. It seems reasonable, therefore, to speculate that polyanionic macromolecules might have the property of reducing supercoiling in nucleoproteins. There is direct experimental evidence to indicate that polyanions can cause increased transcription from chromatin and that non-histone proteins, in particular, can perform this function¹⁴.

Unwinding Mechanism

The first postulate I wish to propose, therefore, is that regions in the eukaryotic genome, which are to be transcribed, contain controlling elements similar to those in bacteria. In addition, closely linked to the promoter locus, each region contains a specific configuration (address locus) to which a destabilizing polyanionic molecule, possibly non-histone protein, can bind to cause a localized reduction of supercoiling. (The DNA configuration need not be highly specific; it could, for example, be no more than a high GC or high AT region. Only a single molecular species of destabilizer need be proposed but a family of related molecules could also be accommodated.) This permits the approach of an RNA polymerase molecule to the promoter, whereas in tightly supercoiled nucleoprotein this is not possible. If control systems, similar to bacterial control systems, operated in eukaryotic cells, one would represent this arrangement diagrammatically as in Fig. 1. If an RNA polymerase molecule attached at the promoter site but failed to pass the regulator loci, only a very small unwound region would be evident. But, if the polymerase molecule reached the initiation site, it could cause further unwinding of the adjacent compact nucleohistone region by accumulation of nascent RNA (also a polyanion) and by the accumulation of an RNA binding protein (Fig. 2).

A mechanism of this kind could provide an explanation for the appearance of puffs in giant chromosomes and loops in lampbrush chromosomes, a requirement for non-histone proteins, the banding pattern of eukaryotic chromosomes, the occurrence of repetitive sequences in eukaryotic DNA (address sites) and also the closed circles formed by self-annealing of eukaryotic DNA¹⁷.

Reinforcement by Gene Reduplication

As it stands this model is inadequate in several respects. In particular, it predicts inactive interband regions which would be too small to recognize, and it does not explain either the large size of loops and puffs or the synthesis of high molecular weight RNA. It is unclear why the transcriptional units in eukaryotes are so large; the explanation may simply have to do with the dimensions or supercoiled or folded nucleoprotein structures, as it is unlikely that a single destabilizing protein molecule of 20,000–50,000 MW could bind to a length of DNA greater than 50–100 Å, which would constitute less than one third of one supercoil of diameter 120 Å; moreover, coiling and uncoiling often behave as cooperative phenomena. Factors of this kind might dictate the need for more address sites which could be met by gene reduplication. There may

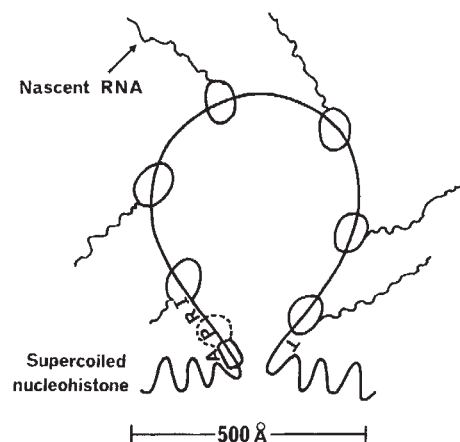


Fig. 2 Structure of a region during transcription. RNA polymerase and nascent RNA induce further unwinding of nucleohistone and permit transcription of the entire structural gene until a terminator (T) is reached.

be other reasons for large transcription units but, whatever the reason, I suggest that the evidence for such a requirement is strong, and that it is most likely to have been met, in the first instance, by gene reduplication. A more realistic model of a transcribable region might be that illustrated in Fig. 3a.

One can see an immediate resemblance between this model and the structure proposed for the ribosomal gene and demonstrated by electron microscopy¹⁸. In this region, sequences specifying ribosomal precursor RNA alternate regularly with sequences of unknown function, called spacers. Could spacers include address loci? There is some evidence that the DNA sequences specifying 5S RNA and those specifying histone messenger RNA are similarly clustered, and these may also have spacer regions^{19,20}.

If there were sufficiently strong selective pressures to maintain this arrangement (for example, a large requirement for

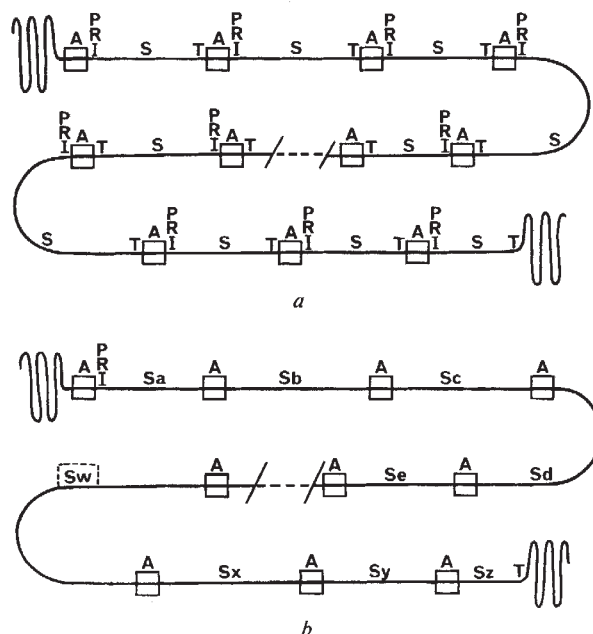


Fig. 3 *a*, The configuration of a region arising by simple gene reduplication. Address loci (A) with associated destabilizers alternate with cistrons each containing promoter (P), regulator (R) and initiator (I) loci, structural information (S) and a terminator locus (T). *b*, The configuration of a fully evolved region. Promoter, regulator and initiator loci occur only at the beginning and a terminator at the end of the region. Address loci may be distributed throughout the region as shown, or clustered at the beginning. The locus contains one "sensible" gene (Sw) and many other sequences (Sa, Sb, etc.) derived from the ancestral gene.

the gene product, and stringent constraints in its structure) it could persist in precisely this form. If we consider a different case, in which there is no strong selective pressure to retain multiple copies of the reduplicated sequences, in particular the extreme case in which retention of only one copy of a structural gene is demanded, we can predict, assuming random mutation, that the other copies would either be lost, or that the nucleotide sequences would diverge progressively by random substitutions until, after about 20% of changes had accumulated, they behave as if unique²¹. The composition of the address loci could not, however, be allowed to diverge beyond limits which would let them bind destabilizers, and their numbers could not be permitted to fall below critical levels. Several possible arrangements might result. If the redundant DNA were lost during evolution, one possible conformation would be a cluster of address sites followed by promoter, regulatory and initiation sites, a single copy of the structural gene and a termination site. If, however, a critical size were required for an effective transcriptional unit (perhaps because the process of transcription itself reinforces the unwinding of nucleoprotein by producing polyanions) some of the "nonsense" DNA sequences would have to be retained. This would result in redundant DNA and redundant RNA.

Among the results of random mutation, promoter, initiator and termination sites could disappear, provided that at least one promoter and one initiator were retained at the beginning of a region and a termination site was retained at the end. This would not necessarily confer any disadvantage, as RNA would still be transcribed in the necessary amounts, although in larger individual molecules; it would also enable initiator sites and promoter sites to disappear. The consequence of this process would be as follows (Fig. 3b). A transcribable region of DNA would have address, promoter and possibly regulator and initiator sites at the beginning. There would follow a series of nucleotide sequences derived from the ancestral genes, only one part of which would carry information to be used for translation, with a terminator. If the whole structure were transcribed, the result would be recognized as an HnRNA molecule. Obviously, there are many other possible variations of this model.

The production of "nonsense RNA" would dictate a need for a "processing" system to prevent unwanted RNA from reaching the translational machinery which it might seriously disorganize (for example, by pre-empting ribosomes); the existence of such processing systems is now generally accepted.

If we postulate that the processing machinery has two essential components, one of which is a nuclease, the other component, which must be predicted, is a signal which will distinguish between RNA molecules to be degraded and those to be preserved. If a component of this "immunity" signal were a nucleotide sequence in RNA, then mutations which resulted in a loss of this structure would result in that RNA being processed. A suitable source of the "immunity" signal might be the secondary structure which exists in rRNA and tRNA and probably exists in globin mRNA²². Since this demands base-pairing between two complementary strands, many mutations resulting in a loss of "sense" would also result in a weakening of secondary structure. This would provide a selective mechanism whereby mutations in sequences specifying special products would result in a failure of the RNA to reach the cytoplasm.

Evolutionary Implications

There are some interesting features of this model from an evolutionary point of view. The first is, of course, that a large pool of nucleotide sequences, ancestrally related to structural genes, would be available for the emergence of new structures. A second is that the mechanism described would offer a rather precise arrangement for controlling gene dosage. Since a large number of "sensible" gene copies would be present initially which would then be reduced by mutation, the organism could

select for the best number of "sensible" copies. A third point is that, according to this model, much of the repetitious DNA would be derived from DNA which originated from structural genes, as is suggested by nearest neighbour frequency studies²³.

This model differs in some respects from that published by Francis Crick¹³, which proposes that single-stranded recognition sites exist at hairpin bends in compact nucleohistone. He postulates an unwinding sequence to help localize the unwinding; this is, in principle, identical to the notion of "address sites" postulated here, but the present model does not necessarily demand single-stranded regions. Perhaps most important is that Crick's model postulates that transcription moves from the compact band regions to interband regions, whereas this model proposes that the interband regions are essentially polymerase-binding sites, and that transcription moves into the band regions from initiation sites which are likely to be situated near the band-interband junctions.

The model is based on the speculation that RNA polymerase (and possibly other molecules) cannot readily bind directly to DNA in the compact nucleoprotein found in the band regions of the chromosome. It postulates "address loci", with relatively non-specific characteristics, which bind destabilizers (probably non-histone protein) in the interband regions thereby permitting polymerase molecules access to DNA. The model assumes a need for a critical size of transcriptional unit; this is postulated to require gene reduplication, which may permit the evolution of structures similar, on the one hand, to ribosomal genes and, on the other, to some of the large lampbrush loops. The model explains the requirements for synthesis of HnRNA and for its subsequent processing.

There is, of course, much more evidence which could be discussed in relation to this model but which has not been cited here. Some ideas have originated in discussions with colleagues; in particular, I believe that the idea that many transcribed sequences might have no informational function originated with Peter Walker.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Detection of Molecular Oxygen in the Martian Atmosphere

MOLECULAR oxygen (O_2) has been detected spectroscopically in the atmosphere of Mars, using lines in the 7620 Å A-band. Weak lines in the wings of the strong terrestrial atmospheric $^oQ(7)$, $^oP(7)$, $^oQ(9)$, and $^oP(9)$ oxygen transitions show equivalent widths of 2 to 4 mÅ. Positive identification of the Martian atmosphere as the source of these lines is based on the following facts: four separate lines are resolved; each line has been observed repeatedly, and in each case it falls at the appropriate predicted Doppler-shifted wavelength¹ with an accuracy of ± 0.01 Å; all four lines are of similar strength as predicted by theory; and the observed lines are not solar Fraunhofer lines (no lines stronger than 0.3 mÅ are observed in the corresponding positions in the Doppler-shifted Venus spectrum).

These observations have been made with the Coudé spectrograph of the McDonald Observatory 268 cm (107 inch) reflector at a dispersion of 0.119 Å/mm (8.4 mm/Å). The rapid spectrum scanner (R. G. Tully, unpublished manuscript) was used in the pulse-counting, echelle mode with GaAs photomultipliers as detectors. The echelle, a Bausch and Lomb 11 × 14 inch blank with 79 grooves per mm, was used in 28th order with a cross-dispersion grating having 300 grooves per mm and blazed at 1 μm. The entire scanner system is under the control of a 'Data General NOVA 1200' computer which moves the scanning mirror and collects the photon counts. A typical scan (approximately 2 Å in length) of one doublet in the oxygen band covers 175 channels in 0.7 s to 3.5 s, depending on the scanning speed. An image rotator allows the disk of Mars to be aligned on the spectrograph slit; in this work

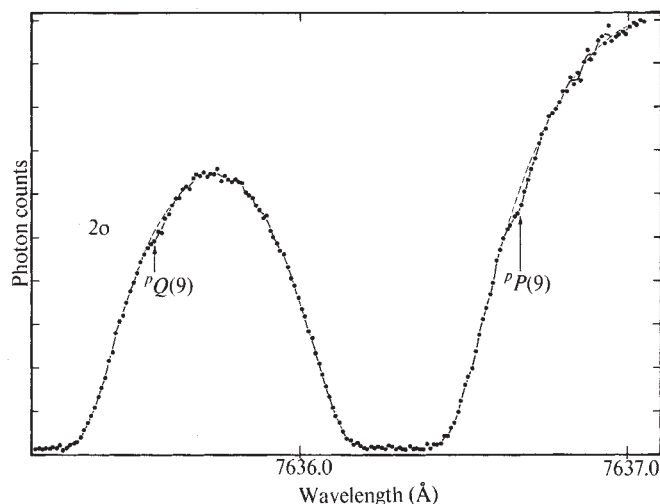


Fig. 2 Fourier transform smoothed scan (solid line) from summation of individual scans (dots) on January 23 of $^oQ(9)$ and $^oP(9)$ lines of the A-band of oxygen. Arrows denote position of Doppler-shifted Martian O_2 lines. Slit resolution was 0.052 mÅ (FWHM). L_{cm} , 283°–317°.

the orientation was pole to pole along the central meridian of the planet. Two spectral slit widths (200 and 400 μm respectively 0.025 Å and 0.050 Å) were used, depending on quantum efficiency of the RCA photomultiplier in operation. These slit widths corresponded to meridional strips of about 0.5 and 1.0 arcs on the disk of Mars. Measured resolutions, using an Fe line with an intrinsic width of 0.026 Å produced by an Fe-Ne hollow cathode tube, were 0.028 Å and 0.052 Å (FWHM).

Detection of such weak Martian lines in the proximity of very strong terrestrial lines is possible because of substantial Doppler shifts produced by the relative orbital motions of Earth and Mars. Observations were obtained on six dates from December 21, 1971, to February 27, 1972; during this period the Doppler shift varied slightly around a mean of +0.38 Å.

During each observing session several spectra were obtained, each of about 30 to 60 min duration. These were combined into a single spectrum for each night before processing through a fast-Fourier-transform smoothing program. Two of the resulting smoothed scans, showing all four lines, are presented in Figs. 1 and 2. The Martian O_2 lines are clearly resolved, with signal-to-noise adequate to permit measurement of the equivalent widths with respect to the local continuum.

Values of the average Martian air mass were calculated as a function of the seeing and of the dimensions of the disk and projected slit (Woodman and Barker, manuscript in preparation). Errors in the measured abundances arise mainly from uncertainty in the shape of the Martian O_2 feature and in the level and placement of the continuum. The O_2 abundances were calculated using line strengths adjusted to 200 K given by Burch *et al.*² and would be about 10% smaller if the line strengths of Miller *et al.*³ were used. Table 1 summarizes the abundances measured for each line.

The average observed O_2 abundance of 9.5 ± 0.6 cm atmosphere is somewhat smaller than the upper limit of 15 cm

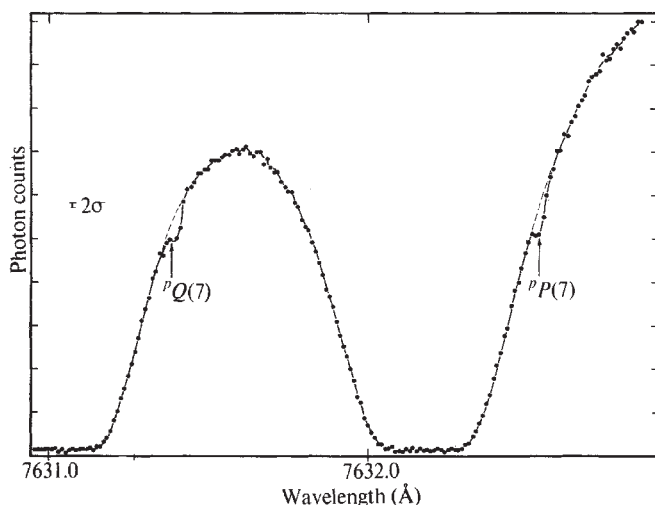


Fig. 1 Fourier transform smoothed scan (solid line) from summation of individual scans (dots) on December 21 of $^oQ(7)$ and $^oP(7)$ lines of the A-band of oxygen. Arrows denote position of Doppler-shifted Martian O_2 lines. Slit resolution was 0.028 mÅ. L_{cm} , 223°–289°.

atmosphere given by Margolis *et al.*⁴ and considerably smaller than the 26 cm atmosphere reported by Belton and Hunten⁵. The observed amount is generally consistent with, although slightly larger than, that predicted using a simplified argument presented by Margolis *et al.* based on photo-dissociation of CO₂ as the primary source of O₂. Based on an observed CO abundance of 47 cm atmosphere⁶ the Margolis argument predicts a molecular oxygen abundance of about 8 cm atmosphere. The observed abundance of 9.5 cm atmosphere implies an O₂/CO₂ mixing ratio of 1.3×10^{-3} (assuming a 5.5 mbar CO₂ atmosphere).

Table 1 Martian O₂ Abundances

Line	Abundance (cm atmosphere)	Number of scans	L_{cm} (°)
⁹ Q(7)	10.9 ± 0.5	9	223–320
⁹ P(7)	8.8 ± 0.4	9	223–320
⁹ Q(9)	9.3 ± 0.9	11	217–317
⁹ P(9)	7.4 ± 1.3	11	217–317

Wt. ave. 9.5 ± 0.6 (s.d. of mean).

The 1971 Martian dust storm had cleared significantly by the time of the observations summarized in Table 1. The abundance reported here may still be slightly smaller than the true abundance, if the residual dust content during these observations was significant over the Martian longitude regions 210° to 320°. Two earlier observations of the ⁹Q(9) and ⁹P(9) lines were made on October 22 and December 1, 1971, during the dust storm. The average O₂ abundances observed on these dates were only 2.4 ± 1 and 7.6 ± 1 respectively, consistent with the line of sight penetrating only the upper Martian atmosphere. Although the lines are much weaker, the scans are of similar quality to those of Figs. 1 and 2, and clearly show the Martian features. The nonuniformity of dust coverage as it cleared precludes any attempt to use the present data to determine apparent variation of O₂ strength with respect to position on the planet's surface.

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Mascons and Isostasy

POSITIVE gravity anomalies have been measured over the circular maria on the Moon¹ and have been taken as evidence that the Moon is not in isostatic equilibrium and must have great strength to great depths. It is further argued that this implies a cold origin for the Moon². Muller and Sjogren¹ adjusted their measurements to give the gravitational field at a height of 100 km above the lunar surface by making reasonable assumptions about the field gradient. They applied no correction to take account of the topography of the surface and none to allow for isostasy. Their published map therefore shows "free air" anomalies. Here

I show that the large, positive free air anomalies can be consistent with complete isostatic adjustment of the circular maria and, consequently, that the Moon may be warm at relatively shallow depths.

If hydrostatic stress can be supported only below some depth in the Moon then isostasy will prevail over regions which are of large horizontal extent compared with this depth, provided that the outer layer is not extraordinarily strong. O'Keefe³ estimated the Bouguer anomalies for the topography on the nearside of the Moon, and found that these are negative and larger than the free air anomalies of Muller and Sjogren, as is observed on Earth. It is taken as evidence that the effects of topography on the gravitational field are compensated by the mass distribution below the surface. If the excess mass of the topography is exactly compensated for, then isostatic equilibrium exists. O'Keefe therefore had good evidence that in general the surface of the Moon is close to being in isostatic equilibrium on the scale of mountain ranges; this implies that the outer layer of the Moon is less dense than the material below it.

The observation that the Moon is not in hydrostatic equilibrium on a large scale can be reconciled with a Moon which is in isostatic equilibrium on a small scale if, for example, the non-equilibrium shape is caused by internal convection⁴. O'Keefe noted that the free air anomalies over the circular maria are smaller than the largest Bouguer anomalies and supposed that the maria were almost but not quite in isostatic equilibrium. It is hard to explain why the maria are not much closer to being in equilibrium. A possible reason is that they have not had sufficient time to adjust since their formation; the time for adjustment to take place can be estimated from Haskell's formula⁵ and, unless either the viscosity or the strength of the lunar material is implausibly high, this time is very short compared with the ages of the maria. The circular maria are therefore likely to be very close to a state of isostatic equilibrium.

A positive free air and gravity anomaly indicates that there is an excess of mass near the surface below the point of measurement. For isostatic equilibrium this excess must be balanced by a deficiency of mass somewhere above the depth at which compensation is complete. There is a mass deficiency above some of the maria because their surfaces are lower than those of their surroundings, and if this were the only deficiency and were exactly compensated by the excess mass in the maria a negative free air anomaly would be seen. The deficiency of mass would be nearer to the point of measurement than the excess and so would have more effect on the gravitational field there; to produce a positive free air anomaly the great majority of the deficiency of mass must lie below the excess mass.

That situation could be produced as follows: I assume that the Moon has a surface layer less dense than its interior, that the maria are layers of lava set into the Moon's surface layer and that the average density of the lava is greater than that of the surface material. A mare then represents a mass excess. If the weight of the lava causes the surface layer to sink into the denser interior material until the buoyancy of the surface balances the extra weight, isostatic equilibrium is achieved and the intrusion of the surface material into the denser interior material gives a deficiency of mass below the excess of mass.

A mechanism for the formation of such a structure on the Moon can be imagined. It is possible that the circular maria were formed by impact and, if this is so, the lava fills did not appear until long after the impacts. In the time between the impact and the appearance of lava a mare crater would adjust isostatically⁶ and its floor would be raised. For a large crater the thickness of the remaining surface layer would be small compared with the diameter of the crater and it would be easily fractured during the uplift. This fracturing would allow lava to escape to the

surface and thus the time between crater formation and infilling would be determined by the rate of isostatic readjustment. The production of lava could have been a result of the thermal evolution of the Moon or it could have been linked to the formation of the mare basin. The removal by impact of a large amount of surface material would have reduced the pressure on the interior material which could then have melted. As a basin filled with lava the floor would sink again and would eventually return to where it was immediately after the impact. If more lava were erupted the basin floor sinks even more, thus pushing light surface material into the denser interior. As the lava erupted there would probably be some slumping of the upper layer because of the removal of material from beneath it; this would occur relatively quickly after eruption and it is unlikely that it would produce isostatic equilibrium. After the lava ceased erupting the structure would continue to adjust isostatically until it achieved equilibrium, involving the flow of material in the Moon's interior.

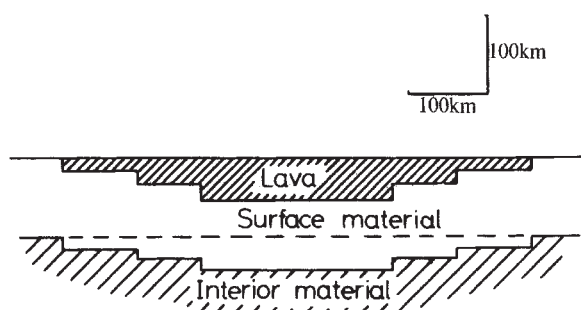


Fig. 1 Model of Mare Serenitatis showing the arrangement of disks for the calculation of the gravity anomaly. ---, Upper bound of the effective mass deficiency.

I have constructed a model mare of this type represented approximately by a set of disks and have calculated the gravity anomalies produced. The curvature of the Moon was neglected although it would have a noticeable effect for a large mare. The gravity field due to a thin disk can be calculated by the method described by Nettleton⁶, which gives good accuracy when the disk is thin compared with its distance from the point at which the field is required. I found, by trial and error, that I would produce anomalies similar to those observed. A model for Mare Serenitatis is given as an example (Fig. 1). A disk of zero density (0.74 km thick and 294 km radius) was included at the surface to account for the fact that the mare is lower than the surrounding region. The lava was represented by three disks: the top one 16 km thick with a radius of 294 km; the middle one 18 km thick with a radius of 200 km; and the lowest one 20 km thick with a radius of 120 km. Three more disks represented the mass deficiency where the light surface material replaced the denser interior material. I assume sharp density discontinuity between the surface material and the interior of the Moon, although a more gradual change could have been used, and took the density of the surface material as $2.8 \times 10^3 \text{ kg m}^{-3}$ and the density of the lava and the interior to be $3.3 \times 10^3 \text{ kg m}^{-3}$. The discontinuity was put at a depth of 100 km and the three disks of negative effective mass were placed immediately below it. They had thicknesses of 15 km, 13 km and 15 km with the same radii as the corresponding lava disks.

The resulting gravity anomaly measured at 100 km above the surface is given in Fig. 2. For comparison the gravity anomaly observed by Muller and Sjogren along a north-south traverse of Mare Serenitatis is shown⁷.

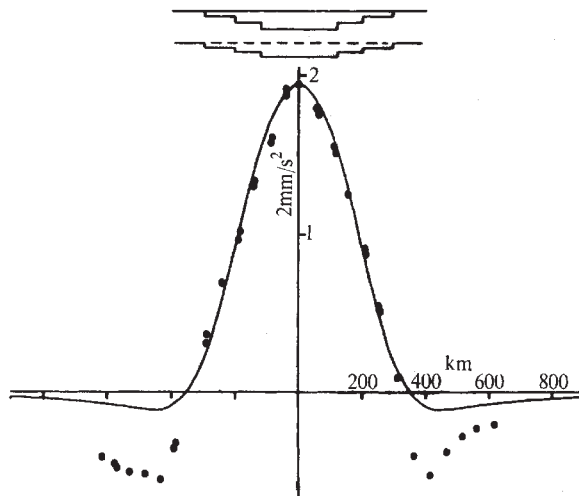


Fig. 2 Gravity anomaly over model of Mare Serenitatis. The anomaly observed by Muller and Sjogren is shown for comparison. ●, Observed; —, model.

The model matches the observations well in the central region and could be modified easily to fit any similar anomaly. Around the edge of the mare the observed anomaly is negative and the model also produces a region of negative anomaly there, although its magnitude is not as large as that observed and is difficult to increase without changing the model appreciably. But it can be seen from the gravity map of Muller and Sjogren that this particular traverse across Mare Serenitatis has exceptionally large negative anomalies and these probably arise from extra structures. If a single concentration of mass, such as a dense sphere or a lava plate, is postulated as the cause of the positive anomaly, some other structure is needed to produce any negative anomaly around the edge of a mare.

The depth of the density discontinuity was chosen arbitrarily. If it had been put deeper, then a thinner layer of lava would have given the same anomaly. The limiting model in this direction is that of a thin plate of lava at the surface of a homogeneous Moon. That would not be in isostatic equilibrium. For isostatic adjustment to take place compensation must occur at a depth less than the horizontal extent of the structure. The change in density must occur above the depth of compensation and so the discontinuity cannot be put deeper than about 200 km. If the discontinuity is brought to the surface then a thicker layer of lava is needed and the limit comes when the discontinuity is at the base of the lava.

The model shows that the positive anomalies observed over the circular maria are entirely consistent with a Moon which can support only hydrostatic stress below a depth of from 70 to 200 km. The question now arises as to whether a structure of the sort described could have survived for at least 3×10^9 yr. A layer of lava similar to the one used in the model would produce a maximum stress difference of about 0.5 kbar in the surface layer. Cold rock would be able to withstand this, but a layer of lava over less dense surface material is an unstable configuration, tending to produce overturning to achieve a stable density gradient. Perturbations of the interface will grow; the rates of growth of small perturbations at a fluid interface are given by Ramberg⁸ for the two-dimensional case. If a layer of lava 50 km thick overlies a layer of surface material 100 km thick, the wavelength of the fastest growing perturbation is about 300 km if both layers have the same viscosity. The characteristic time of growth is 7×10^9 yr if the viscosity is $10^{25} \text{ kg m}^{-1} \text{ s}^{-1}$. This time is approximately proportional to the viscosity of the surface layer material and also depends greatly on the thickness of the layer—it would be doubled if the layer were about 16% thinner.

A three-dimensional treatment would give a slower rate of disruption and if the lava were thicker in the middle this would also probably add to its stability. A plate of uniform thickness would begin to sink at the edge⁸ but thickening at the centre would presumably counteract this. It seems therefore that a lava layer could last long enough.

I have shown that the positive gravity anomalies associated with circular maria do not necessarily imply that a large part of the interior of the Moon is cold. The anomalies could be produced even if the Moon cannot support stress differences below depths of the order of 100 km. Better knowledge of the topography, more detailed gravity surveys and more evidence from seismology will allow the determination of the true structure of the maria.

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Experimental Evidence against the Role of Selective Volatilization on the Lunar Surface

It has been suggested¹ that the deduced sequence of crystallization and observed chemical zoning in plagioclase of a feldspar-rich lunar basalt (Fra Mauro 14310) provides evidence for selective volatilization of sodium and potassium from lunar magmas during eruption and flow at the lunar surface. Previous arguments^{2,3} for volatilization were advanced to explain the absence of plagioclase on or near the liquid of lunar basalts²⁻⁶ and were in part based on alkali loss in experiments with basalt samples maintained at high temperatures in high vacuum. The recent work¹ for the first time adduces evidence for Na and K loss from data directly derived from the rocks themselves, and presents a quantitative estimate of the degree of alkali loss and the postulated magma composition prior to loss of volatiles.

Basalt 14310 is plagioclase-rich, with plagioclase laths ranging in size from phenocrysts over 1 mm to microlaths of less than 10 microns in length. Brown and Peckett¹ have assumed that the plagioclase crystals represent several generations, a sequence of nucleation and growth in which the growth of the large phenocrysts was followed by nucleation of the groundmass laths and then by the growth of microlaths. Because the composition of plagioclase reflects the composition of the liquid from which it crystallized, Brown and Peckett use the plagioclase compositions to infer liquid compositions. They state (p. 263): "The phenocrysts are strongly zoned to more sodic and potassic compositions whereas the abundant groundmass laths and even the microlaths in the residual mesostasis are as low in sodium and potassium as the phenocryst cores. In view of these data, we propose that the basalt lavas lost Na and K on the lunar surface". They then use the analyses of plagioclase phenocryst rims and modal plagioclase content to estimate that, prior to volatile loss, the magma contained 3.14% Na₂O

and 0.70% K₂O (compare with Table 1), and conclude that minimal alkali loss was 74% Na₂O and 68% K₂O and refined estimates could be as high as 86% Na₂O and 78% K₂O.

In Table 1 we list the composition of 14310 and of two glasses prepared by adding Na₂O and K₂O to 14310 according to the hypothesis discussed. If these glasses were indeed parents to 14310, then the latter would have lost 75% Na₂O and 62% K₂O (mix I) or 85% Na₂O and 74% K₂O (mix II). The hypothesis of volatile loss can be tested by determining whether the compositions with the alkalis restored give the same sequence of crystallization and give compositions of phases matching those of the earliest stage of crystallization of the natural rock. If the hypothesis is correct, the compositions with the alkalis restored should first precipitate plagioclase of composition matching that of the phenocryst cores.

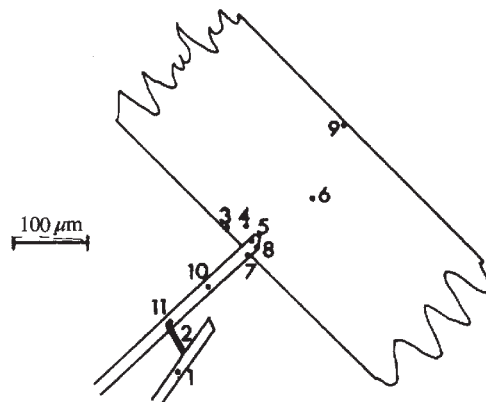


Fig. 1 Sketch illustrating intergrowth of microlaths (2), groundmass laths (10, 11) and phenocrysts (6) and compositions at various points within these crystals. The zoning to sodic plagioclase shown at (3) and (9) is not general at the edge of the phenocryst but in most areas the closest analysable composition at the edge was $> \text{An}_{90}$. Plagioclase (molecular proportions): 1, $\text{An}_{94}, \text{Or}_{0.5}$; 2, $\text{An}_{77}, \text{Or}_{3.2}$; 3, $\text{An}_{72}, \text{Or}_{3.6}$; 4, $\text{An}_{93}, \text{Or}_{0.7}$; 5, $\text{An}_{93}, \text{Or}_{0.6}$; 6, $\text{An}_{95}, \text{Or}_{0.5}$; 7, $\text{An}_{93}, \text{Or}_{0.6}$; 8, $\text{An}_{93}, \text{Or}_{0.7}$; 9, $\text{An}_{71}, \text{Or}_{4.0}$; 10, $\text{An}_{95}, \text{Or}_{0.6}$; 11, $\text{An}_{95}, \text{Or}_{0.5}$.

In Table 2 we present the sequence of phases observed at 1 atmosphere crystallization studies of the compositions listed in Table 1. In 14310 composition, plagioclase, at least as anorthite-rich as An_{92} , is the first phase to appear, and crystallizes alone over 100° C temperature interval. By comparison, the composition of the phenocryst plagioclase in the natural rock 14310 ranges from An_{95} to An_{90} (refs. 1, 7). In alkali-enriched mix I, aluminous spinel is the first phase to crystallize and plagioclase appears only with olivine and spinel at 1,190° C. In this composition, olivine does not react with liquid at around 1,180° C but persists as a major phase to lower temperatures (note the normative compositions of Table 1). Thus the sequence of crystallization differs markedly from that illustrated by the natural rock 14310. Furthermore, the earliest plagioclase to appear is much more sodic (An_{81}) than the phenocryst cores of the natural rock, and this composition (mix I) cannot crystallize plagioclase as anorthite-rich as that observed. Similarly, the spinel accompanying the plagioclase is 84% spinel, 16% chromite, and bears no relation to the natural ulvospinel (84% ulvospinel, 9% spinel, 7% chromite) which is a minor phase in 14310.

In the alkali-enriched mix II, spinel is again the liquidus phase and olivine and spinel crystallize together over 100° C before plagioclase appears. The earliest plagioclase is An_{76} , which is close to the most sodic rims occurring on some phenocrysts in 14310 ($> \text{An}_{67}$ (Fig. 1)). Thus, while a liquid of this composition would indeed crystallize plagioclase as sodic as the most sodic plagioclase phenocryst rims observed in the rock, it cannot precipitate the calcic plagioclase of which the phenocrysts are predominantly composed. The orthoclase content

Table 1 Compositions and CIPW Norms of Compositions Studied Experimentally

	14310 ⁷	Alkali-enriched * mix I	Alkali-enriched * mix II	KREEP ⁷
SiO ₂	47.6	45.6	44.4	48.5
TiO ₂	1.2	0.9	0.9	2.0
Al ₂ O ₃	20.7	20.5	19.9	16.7
FeO	8.2	8.8	8.8	11.1
MnO	0.1	0.2	0.2	0.0
MgO	7.6	7.3	7.2	8.3
CaO	12.5	12.4	11.8	11.5
Na ₂ O	0.7	2.8	4.8	0.6
K ₂ O	0.5	1.3	1.9	1.2
Cr ₂ O ₃	0.2	0.2	0.2	—
P ₂ O ₅	0.4	—	—	0.6
$\frac{\text{Ca}}{\text{Ca} + \text{Na}}$ (atomic ratio)	91	71	58	93
CIPW norms				
Qz	0.3	—	—	—
Or	3.0	7.7	—	7.2
Ab	5.9	4.7	—	5.2
An	52.0	39.5	27.1	39.2
Ne	—	10.3	22.0	—
Lc	—	—	8.8	—
Di	6.2	18.1	24.3	11.6
Hy	29.1	—	—	32.0
Ol	—	17.7	15.3	—
Cs	—	—	0.6	—
Ilm	2.3	1.7	1.7	3.8
Ap	1.0	—	—	1.3
Chro	0.3	0.3	0.3	—
Normative plagioclase	An ₈₅ Ab ₁₀ Or ₅	An ₇₆ Ab ₁₀ Or ₁₅	An ₁₀₀	An ₇₆ Ab ₁₆ Or ₁₄

* Compositions determined by electronprobe analysis of prepared glasses.

Table 2 Results of Experimental Study of Melting Relationships * of 14310 and Alkali-enriched Compositions

Compositions	Sequence of crystallization	Composition of plagioclase (mol %)
14310	Plagioclase (1,330° C), olivine (1,220° C), pyroxene (1,180° C) (olivine reacts out at 1,180° C)	An ₉₂ Ab _{7.5} Or _{0.5} (1,320° C)
Alkali-enriched mix I	Spinel (1,250° C), plagioclase, olivine (1,190° C), pyroxene (~1,180° C)	An ₈₁ Ab ₁₈ Or ₁ (1,190° C)
Alkali-enriched mix II	Spinel (1,190° C), olivine (1,170° C), plagioclase (1,080° C)	An _{75.5} Ab _{23.5} Or ₁ (1,080° C)
KREEP	Olivine (1,225° C), plagioclase (1,220° C), pyroxene (1,180° C)	An _{89.5} Ab _{8.5} Or _{2.0} (1,215° C)

* Experiments were carried out in metallic Fe capsules, enclosed in evacuated SiO₂ tubes, suspended in a 1 atmosphere, pt-wound cylindrical furnace^{4,6,7}.

of the central parts of natural phenocrysts is 0.5%. Experiments on the three compositions described and on the KREEP composition⁷ show that the natural phenocrysts precipitated from a liquid with K/Ca and K/Na ratios as in 14310, and could not have precipitated from the other compositions listed in Table 1.

We have re-examined the range of plagioclase compositions occurring in 14310, including samples 14310/13 and 14310/20 analysed by Brown and Peckett¹. We confirm their observations and analytical data, but disagree with their interpretation that the size sequence from phenocryst to microlath was also a sequential order of nucleation and growth. We observed that small laths and even microlaths may penetrate well inside the borders of the large phenocrysts, implying the existence of very small plagioclase crystals prior to growth of outer zones of the phenocrysts. In an example where a small lath penetrated into a phenocryst, variations in plagioclase composition showed that the local precipitation of sodic plagioclase occurred after growth of groundmass plagioclase (see Fig. 1).

We conclude that this rock represents rapid cooling and crystallization of a liquid of the same composition as the present rock⁷. The presence of sodic rims to plagioclase crystals is due to late-stage enrichment in Na and K in local,

interstitial, residual liquids. Our experiments show that the precipitation of plagioclase of the compositions observed in the natural phenocrysts and groundmass requires that the parental liquid have the same Ca/Na and Ca/K ratios as the present rock. The hypothesis that the present rock lost > 60% Na₂O and K₂O during eruption at the lunar surface is shown to be incompatible with the presence of anorthite phenocrysts. The matching of the natural crystallization sequence in 14310, like that of mare basalts previously studied, with those observed experimentally, demonstrates that there has been no quantitative loss of alkalis by vacuum volatilization during eruption of magmas at the lunar surface.

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Optical Polarization of the Supernova in NGC 5253

If a type I supernova is the result of the explosion of a star with a degenerate nucleus (see, for example, Baglin¹) it is likely that a large magnetic field will be present in the radiating region for a short period following the outburst. Depending on the emission process a sufficiently large field could produce a measurable optical circular polarization. For example, for bremsstrahlung in an optically thick but very dilute plasma in an ordered magnetic field of H gauss, the fractional circular polarization in the optical continuum is $q \sim 10^{-9} H$ (see refs. 2 and 3). If the degenerate progenitor of the supernova is entirely destroyed in the explosion, conservation of magnetic flux dictates that q would reach the limit of detectability ($q_{\text{LIM}} \sim 3 \times 10^{-6}$; $H_{\text{LIM}} \sim 3,000$ G) in only 20 s for either a white dwarf (initial radius $r_0 \sim 10^9$ cm, initial field $H_0 \sim 10^6$ G) or a neutron star ($r_0 \sim 10^6$ cm, $H_0 \sim 10^{12}$ G) assuming an expansion velocity of 10^4 km s⁻¹. If a sizable fragment survives, however, a measurable polarization might be seen for a much longer period. For synchrotron emission smaller fields can produce a detectable polarization. According to Legg and Westfold⁴ a hypothetical field $H \sim 10^6$ G in the Crab nebula would produce $q \sim 10\%$ in the visible: since $q \propto H^{1/2}$ a polarization $q_{\text{LIM}} \sim 3 \times 10^{-6}$ corresponds to $H_{\text{LIM}} \sim 10^{-3}$ G. However, the precise conditions strongly influence^{5,6} the relations between q and H ; the interpretation would therefore be uncertain even if this mechanism were shown to be dominant. The influence of radiative transfer in the dense layers near the star and of a possible fluorescence emission in the outer layers of the shell⁷ would make the interpretation for the synchrotron process even more difficult.

We have measured the polarization of the bright supernova discovered by Kowal⁸ on May 13, 1972, in the irregular galaxy NGC 5253. Spectroscopic evidence^{9,10} suggests that it is type I: an earlier supernova (Z Centauri) in this galaxy observed in 1895 was also type I. The fractional linear and circular polarizations measured with no filter ($\lambda\lambda 3500$ to 6500 Å) on June 13.3 (UT), 1972, are respectively: $p = (3.5 \pm 2.0) \times 10^{-3}$, $q = -(0.8 \pm 0.4) \times 10^{-4}$. The linear polarization is comparable with the value measured by Serkowski¹¹ in the supernova in NGC 5253. An explanation for the small upper limit on the circular polarization ($|q| < 10^{-4}$) may be sought in terms of either (1) bremsstrahlung or synchrotron radiation, followed by radiative transfer and fluorescence, or (2) interstellar circular polarization¹². It is unlikely that the latter is involved, because if we assume that the observed linear polarization $p = 0.35\%$ is produced by aligned grains in our Galaxy, then the expected broad band circular polarization is too small, namely $q \approx 0.7 \times 10^{-5}$. (This conclusion is based on data for 6 stars studied by Kemp and Wolstencroft (unpublished).) The upper limits to H for the bremsstrahlung and synchrotron processes obtained from $|q| \leq 10^{-4}$ are 10^5 G and 1 G respectively. (The former value includes allowance for radiative transfer³.) If either of these processes produced the

emission close to the star, then the field was less than 10^5 G at the time of our observation.

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Crust and Mantle of the Gulf of Mexico

A SEEMING paradox has puzzled investigators of the crustal structure of the Gulf of Mexico since Ewing *et al.*¹ calculated that a unit area of the rather thick crust in the gulf contains less mass than does a combination of the crust and enough of the upper mantle to make a comparable thickness in the Atlantic Ocean. They also noted that the free-air gravity of the gulf is essentially normal and fails by a large factor to be low enough to reflect the mass difference that they calculated. We propose a solution to this problem.

The study by Ewing *et al.* was based on comparative summations of thickness multiplied by density for the various layers in each setting. Thickness was derived from seismic-refraction inflexions and density from Nafe and Drake's curve² relating density and seismic velocity. From this crustal analysis and data for the Gulf of Mexico's Sigsbee Deep and the western Atlantic basin¹, the present floor of the gulf should lie at a depth of only 1.9 km, whereas its actual depth is 3.7 km.

Wilhelm and Ewing³ suggest that oceanization may explain an increased crustal density for the Gulf of Mexico. This concept, developed by Belousov⁴, is that mantle material intrudes crust of the continental type, expels volatile components, and converts silicate minerals into denser phases. Crustal products created by such metasomatism, however, should be compatible with the Nafe-Drake curve, so the concept of oceanization does not explain the mass deficiency.

Antoine and Pyle⁵ suggest that turbidity-current deposits in the gulf might have a relatively high density for their seismic velocity, thereby causing the Nafe-Drake relation to give incorrect densities for this area. But, as Menard⁶ points out, the densities required to make the crust of the Gulf of Mexico match the section in the Atlantic Ocean fall outside the entire field of Nafe-Drake data points, which include a wide variety of hard rocks and sediments. Menard concludes that the compensating mass required to provide the balance must lie in the mantle of the Gulf of Mexico. The density of the mantle in the gulf was not included in the calculations of Ewing *et al.*¹, as they considered it to be the same as that of the Atlantic basin.

We propose that the differences are caused partly by differences in depth to the low-velocity interface, which lies in the mantle at a depth of about 60 km, and chiefly by small-temperature differences in the thick layer of mantle above the interface. Owing to sediment loading, the Mohorovičić (M) discontinuity (the top of the mantle) lies at a depth of 18.8 km under the Sigsbee Deep, or about 6 km deeper than in the western Atlantic Ocean. Some of the compensating mass is

believed to occur where the low-velocity interface is depressed to match the 6 km depression of the M discontinuity. Because the material above the low-velocity interface has a seismic velocity about 0.2 km/s higher than that below it⁷, applying a differential density of about 0.2 g/ml. from the Nafe-Drake curve to a 6 km interval at the low-velocity interface partly reduces the seeming discrepancy.

The remaining and largest part of the apparent difference in mass is believed to be related to a greater age of the Gulf of Mexico basin (Jurassic) compared with the average for the western Atlantic basin (Cretaceous). Sclater *et al.*⁸ have shown that thermal contraction resulting from dissipation of initial heat after sea-floor spreading causes mid-ocean rises to sag more than 3 km during the 10⁸ yr required to approach a new thermal equilibrium. A 0.1 g/ml.³ difference in density caused by such thermal contraction in the 40–50 km layer between the M discontinuity and the low-velocity interface of the Gulf of Mexico and the Atlantic basins would be more than enough to balance the two sections. It would be reflected by a velocity difference of no more than 0.1 km/s, which is within the range of reported differences. Hence, a slightly cooler and therefore denser upper mantle (owing to closer attainment of thermal equilibrium), commonly accompanied by a thicker crust caused by greater sedimentation, may provide the principal lithospheric contrasts between older small ocean basins such as the Gulf of Mexico and the younger parts of large ocean basins.

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BIOLOGICAL SCIENCES

Frequencies of Amylase Variants in *Drosophila melanogaster*

THE selective significance of enzyme polymorphisms remains unsettled^{1,2}. The frequencies of some enzyme variants are correlated with environmental factors³, which might cause

frequency changes by acting on closely linked genes. We therefore studied amylase variants in *Drosophila melanogaster*. A major function of amylase in *Drosophila* is in the digestion of starch. Genetical and physiological investigations on amylase variants were made by Kikkawa⁴, Doane^{5,6} and Bahn⁷. Using electrophoresis they found five main bands of amylase activity. In homozygous flies one or two bands are present, caused by two closely linked genes on the second chromosome (2–78.1), presumably originated by duplication. Amylase activity of the variants differs on the same food medium, and also depends on the composition of the food⁶. Banding patterns in larvae and in adult flies are identical (ref. 6, and A. J. W. H., unpublished work).

We studied four cage populations based on flies from widely different geographic regions: Pacific I and Pacific II from the USA, Kaduna from Africa, Bogota from South America. We used two food media: one with sucrose as the only source of carbohydrate (S-medium: 16 g agar, 45 g sucrose, 27 g yeast, 11 ml. nipagin in 1,000 ml. tap water) and one with cornmeal as a main component (C-medium: 8 g agar, 77 g sucrose, 154 g cornmeal, 25 g yeast, 8 ml. nipagin in 1,000 ml. tap water). The populations were kept for at least four years on S-medium before offshoots were made onto C-medium. After the Pacific II S population became extinct, a new Pacific II S population was originated from the C population. The frequencies of the amylase variants were determined when the C populations were 2 yr old and the Pacific II S population 8 months old.

Adult flies were homogenized in 0.025 ml. 0.1 M phosphate buffer, pH 7.5. Electrophoresis was for 3 h on 0.9% agarose in 41 mM veronal buffer, pH 8.4, at 14 V cm⁻¹. Gels were incubated in a 0.2% boiled starch solution (Merck's soluble starch) in phosphate buffer, 0.025 M, pH 6.7, at 36° C for 45 min. Staining was done in I-KI solution (1 M) for 15 min.

Amylase genotypes cannot be determined unequivocally on the basis of the banding pattern. Patterns 1–3 can represent *amy1,3/amy1,3*, *amy1,3/amy1*, *amy1,3/amy3* or *amy1/amy3*. Similar considerations can be applied to 1–2 and 4–6. Presence and absence of combinations of bands and allelic behaviour of patterns show that 4–6 always represents *amy4,6/amy4,6* while 1–2 and 1–3 can represent both homozygotes (*amy1,2/amy1,2* and *amy1,3/amy1,3*) and heterozygotes (*amy1,2/amy1,3/amy1*). Table 1 shows the frequencies of amylase types in the eight populations. Striking differences in phenotype frequencies are found between populations of the same geographical origin on different food media. Although there are differences in frequency between populations of different geographic origin on the same medium the change in frequency between media is in the same direction in all four populations.

If we assume that our amylase variants have the same activity levels as the corresponding variants of Doane⁵, the variant *amy4,6* that has increased on cornmeal medium

Table 1 Frequencies of Amylase Variants in Four Populations on Two Media

Population medium:	Pacific I		Pacific II		Kaduna		Bogota	
	Cornmeal	Sucrose	Cornmeal	Sucrose	Cornmeal	Sucrose	Cornmeal	Sucrose
<i>amy4,6/amy4,6</i>	0.539	0.263	0.322	0.232	0.332	0.073	0.122	—
<i>amy1,3/amy1,3</i>	0.061	0.199	0.115	0.151	0.088	0.336	0.169	0.444
<i>amy1,3/amy1</i>	0.004	0.076	0.021	0.007	0.059	0.260	0.272	0.556
<i>amy1,2/amy1,2</i>	0.025	0.006	0.035	0.103	—	0.021	—	—
<i>amy1,2/amy1</i>	0.216	0.314	0.185	0.202	0.218	0.093	0.091	—
<i>amy1,3/amy4,6</i>	0.78	0.003	0.185	0.162	—	0.003	—	—
<i>amy1,2/amy4,6</i>	0.069	0.139	0.108	0.110	0.303	0.215	0.347	—
<i>amy1,3/amy1,2</i>	0.008	—	0.028	0.033	—	—	—	—
Number of flies scored	245	331	286	272	238	289	254	275
X ² between media	X ² = 488 7df P < 0.001		X ² = 25.445 7df P < 0.001		X ² = 139 5df P < 0.001		X ² = 498 4df P < 0.001	

Eggs were collected from the cages; 200 eggs were transferred to 1/8 litre culture bottles with 40 ml. of the appropriate food; each frequency is based on equal numbers of males and females.

containing starch has the highest activity. It seems probable that this variant confers selective advantage because individuals carrying it can exploit food with starch more effectively than individuals carrying variants with lower activity.

Recombination is shown to occur between the two loci involved^{5,7}, and a number of other banding patterns, like *amy*3,4 and *amy*1,6, are probable. Obviously deviation from linkage equilibrium is complete in all populations. This is an additional indication for the action of selection. The low recombination frequency between the loci involved (0.008%) offers a favourable situation for the generation of linkage disequilibrium. In view of the maintenance of the polymorphism, selection for variants with higher activity on starch medium seems to be balanced by other selective factors.

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Olfactory Bulb Removal but not Anosmia increases Emotionality and Mouse Killing

THE extent to which olfactory stimuli control animal behaviour is a current topic of empirical investigation and theoretical speculation. In many studies a critical experimental strategy has been the complete elimination of olfactory sensation, achieved by surgical removal or destruction of the olfactory bulbs. Considerable disruptions of sexual¹⁻⁴, maternal⁵, territorial^{6,7} and aggressive^{8,9} activities occur after centrally induced anosmia in various species. According to some reports, bulbectomy also induces irritability and hyperemotionality resembling the classic septal "rage" syndrome¹⁰⁻¹³.

These data present interpretive difficulties because it may be erroneous to identify loss of olfaction as the sole cause of behavioural alterations after bulbectomy. A method for producing an anosmic animal without removing the olfactory bulbs would help to distinguish between those behavioural changes resulting from anosmia and other non-olfactory effects of olfactory bulb removal. Alberts and Galef recently demon-

strated with a behavioural test that a single intranasal treatment with zinc sulphate induces anosmia in the rat¹⁴, apparently related to coagulation necrosis of cells in the olfactory epithelium¹⁵⁻¹⁷. Thus it is possible to study an experimentally anosmic animal with an intact central nervous system. We now report that rats rendered anosmic by intranasal zinc sulphate and bulbectomized-anosmic rats are behaviourally disparate, and that destruction of central nervous tissue, not anosmia, seems to mediate both increased irritability and mouse killing behaviours exhibited by bulbectomized rats.

After at least 1 week of periodic handling and habituation to individual cages, a hundred adult female Sprague-Dawley rats were assigned randomly to treatment groups. Baseline levels of emotionality for each rat were established on the day preceding the first operative treatment. A 0-3 ordinal index, similar to emotionality scales used previously^{18,19}, was administered by an assistant unaware of the group assignment of any rat.

Thirty rats were given three intranasal treatments with zinc sulphate, spaced 5 days apart; twenty animals received intranasal treatments with physiological saline. The ZnSO₄ solution used was isotonic with normal body fluids (7.65%). The 23 gauge hooked catheter measured 2.5 × 3.5 × 4.2 mm and is smaller than the catheter originally described by Alberts and Galef¹⁴. Multiple zinc sulphate treatments were used to maintain anosmia because the effects of a single treatment are usually not permanent¹⁴. One day elapsed between the first treatment and post-operative testing. Thirty rats sustained bilateral olfactory bulb removal by aspiration under nembutal anaesthesia and twenty underwent sham bulbectomy. These animals were left for 3 days before testing was resumed. Post-operative testing was conducted for 2 weeks and consisted of three additional emotionality tests for each rat and concomitant recording of mouse killing behaviour. Mice were housed continuously with all rats after the first post-operative test.

Eight rats in each experimental group (bulbectomy and zinc sulphate) were reduced to 80% body weight and trained to perform a simple olfactory discrimination finding palatable food. Before experimental treatments the trained rats correctly discriminated between two identical wood-chip-filled cups, positioned randomly, one of which contained accessible Noyes pellets; a layer of Purina chow was pressed below a perforated false bottom as an additional food odour. Pre-operative levels were 92-100% correct responses, decreasing to chance levels post-operatively (twelve trials per day) for all rats. Loss of discriminative ability confirmed the olfactory deficit. These rats had been screened for mouse killing during 5 weeks of cyclic food deprivation and were confirmed non-killers before experimentation. Fully fed mice were housed with the food-deprived rats to avoid competition over the daily ration of Purina pellets.

The results are summarized in Table 1. Bulbectomized-anosmic rats became hyperemotional according to our rating scale. In the final post-operative test 63% of the bulbectomized animals increased in overall emotionality, relative to their own pre-operative baseline. Reactivity to handling was the single measure reflecting the greatest differences within our scale.

Table 1 Summary of Experimental Results for all Subjects (N=100)

Treatment	Mouse killing	Increased emotionality on final post-operative test	Increased reactivity to handling on post-operative tests		
			Test 1	Test 2	Test 3
Zinc sulphate	10% (3/30)	20% (6/30)	10% (3/30)	10% (3/30)	16% (5/30)
Saline	0% (0/20)	20% (4/20)	10% (2/20)	10% (2/20)	0% (0/20)
Bulbectomy	53% (16/30)*	63% (19/30)*	43% (13/30)†	30% (10/30)‡	56% (17/30)*
Sham bulbectomy	10% (2/20)	25% (5/20)	10% (2/20)	15% (3/20)	15% (3/20)

Changes in emotionality and reactivity to handling are expressed as increases (percentage of group and frequency), relative to each rat's individual pre-operative baseline. Levels of significance (χ^2 test): * $P < 0.001$; † $P < 0.01$; ‡ $P < 0.1$.

Fifty-three per cent of the bulbectomized rats killed mice, including four of the eight rats previously screened for non-killing. These data are in exact accord with previous findings^{8,20}. Inspection of whole perfused brains indicated that the damage produced by our bulbectomies ranged from discrete removal of the rostral portions of the olfactory bulbs to extensive damage including the olfactory peduncles and portions of the tubercle. Ranked correlation of magnitude of tissue damage to measured irritability was 0.24 ($n=18$).

Repeated zinc sulphate treatments induced and maintained anosmia for the 2 week test period but, in contrast to olfactory bulb ablation, increased neither emotionality nor mouse killing. The performance of these animals did not differ significantly from either control group.

Thus, our data indicate that olfactory bulb removal can induce behavioural changes in rats which are not a consequence of the olfactory deficit alone. Other authors¹⁰ have interpreted the hyperemotionality exhibited by bulbectomized rats to be a result of "psychological" stress related to loss of olfactory information from the environment, a conclusion contradicted by the present study. Similarly, the emergence of mouse-killing in bulbectomized rats cannot be attributed to anosmia.

Although zinc-sulphate induced anosmia failed to alter the behaviours measured in our study, this treatment has been shown to exert other behavioural changes. For example, it can eliminate territorial aggression in wild rats (Alberts and Galef, in preparation). Fleming and Rosenblatt recently reported that each of the techniques can produce significant and divergent effects: bulbectomy can eliminate and zinc sulphate potentiate pup-induced maternal behaviour in virgin rats (findings presented at American Psychological Association meeting, 1972).

Our findings also bear directly on several current research issues unconnected with olfactory control of behaviour. Deficits in non-olfactory learning tasks²¹⁻²³, heightened sensitivity to some exteroceptive stimuli²⁴, changes in ingestive behaviours^{25,26}, chronic alterations in central catecholamine levels²⁷ and disruptions of reproductive physiology^{28,29} have been reported after bulbectomy. These effects may be the result of anosmia (the sensory deficit) or some other consequence of olfactory bulb ablation related, perhaps, to interruption of a non-sensory component of the "olfactory" system. It is clear, however, that "anosmic" and "bulbectomized" are not conceptually synonymous and should, in the future, be carefully distinguished.

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Evidence for Ancient Tetraploidy and Conservation of Linkage Groups in Mammalian Chromosomes

OHNO¹⁻³ has suggested that during mammalian evolution tetraploidy occurred with a resultant doubling of the chromosome number and DNA content, from $2-3 \times 10^8$ yr ago¹⁻⁴. The recent technique of chromosome banding, through the use of quinacrine fluorescence and giemsa staining, has allowed evidence such as similar pairs of chromosome pairs in the karyotypes of modern mammals to be examined. The following observations are compatible with the existence of modern remnants of ancient tetraploidy.

1. The human karyotype is readily divisible into pairs of pairs (Fig. 1). Similar pairs of whole chromosomes include 1 and 2, 4 and 5, 7 and 8, 11 and 12, 14 and 15, 16 and 17, 19 and 20, and 21 and 22. The two arms of chromosome 3 (3p+3q) are similar, suggesting that ancestral homologues have formed a metacentric chromosome by Robertsonian

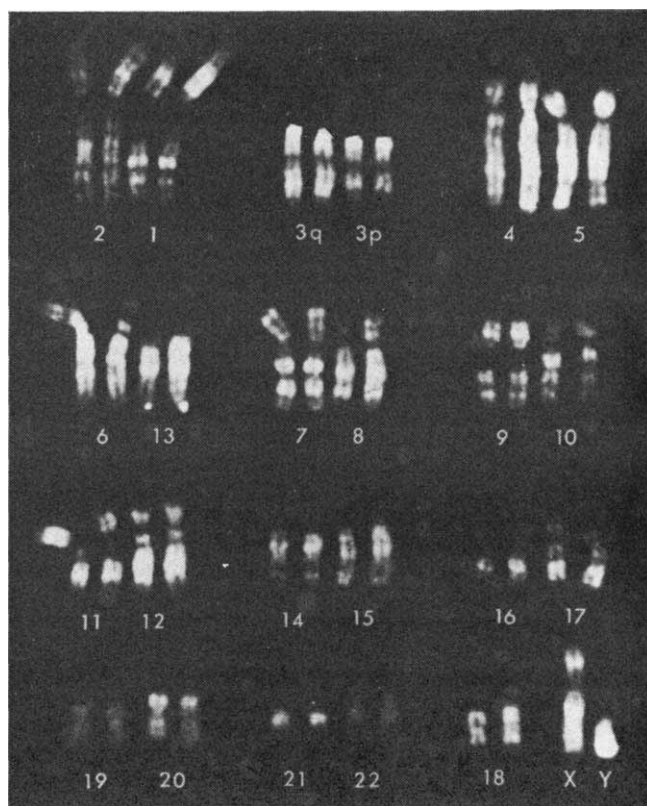


Fig. 1 Rearrangement of the human karyotype into sets of fours, reflecting possible ancestral homologues (see text). (Original quinacrine fluorescence karyotype courtesy of Drs Lin and Uchida.)

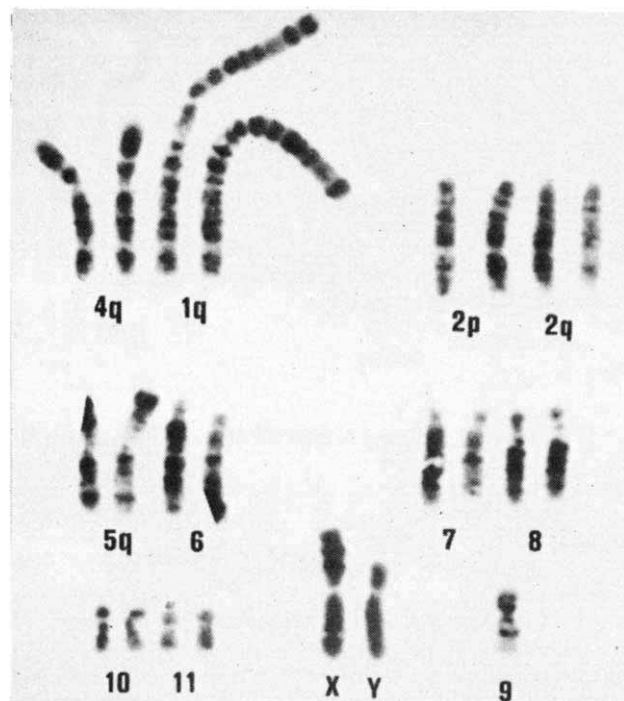


Fig. 2 Rearrangement of the karyotype of the Chinese hamster into sets of fours, reflecting possible ancestral homologues. The chromosomes are from a Don line in which the other homologue of chromosome 9 is missing. (Original trypsin banded karyotype courtesy of Dr Stubblefield.)

fusion. A paracentric inversion in 9 or 10 would make the banding pattern of these two pairs almost identical. The inverted arms of chromosome 13 resemble 6q. The long arm of chromosome 18 is similar to Xq. Some alternative arrangements are also possible. For example, 20p and 21q might be ancient homologues.

2. Several of the Chinese hamster chromosomes can be placed in sets of four (Fig. 2). These include the distal portion of 1q and 4q, 2p and 2q, 5q and 6, 7 and 8, and 10 and 11.

3. Although the giemsa banded chromosomes of the mouse⁵ can be grouped into sets of four, several alternative arrangements are possible. Studies of the chromosomes of *Mus poschiavinus*, in which 14 acrocentrics have fused to form 7 metacentrics, suggest that several of the fusion chromosomes may involve acrocentrics that were ancient homologues. This is seen by the tendency for the patterns in the arms of the metacentrics composed of chromosomes 6 and 5, and 14 and 19, to mirror each other (see ref. 6).

4. In the Indian muntjac, the fluorescent patterns in the arms of the large metacentric number 1 chromosome show many similarities⁷, suggesting that this chromosome may have been formed by the fusion of an ancestrally related pair of homologues.

A valid criticism of these points is that, given any random series of dark and light bands, there is a good possibility of lining them up into any type of relationship one desires. I suggest, however, that the ease of pairing, especially in humans, seems beyond the realm of chance, and there is some ancillary evidence from studies of gene linkage (see below). Also the implications of the theory are so wide ranging that it is worth stating the hypothesis to encourage its being tested.

I suggest that ancestral linkage groups have been broken down by chromosomal rearrangements much less than previously expected. If homologues dating from a tetraploid event occurring 2×10^8 yr ago are still detectable, general linkage relationships for significant segments of chromosomes have been retained. This would suggest that similar sets of banding patterns might be detectable even between rather distantly related species, and a study of giemsa-banded prometaphase chromosomes from such species would be rewarding.

There is indeed some evidence for similar linkages between certain genes in divergent species; for example, the coat colour mutants, pink-eyed and albino, are approximately 15 crossover units apart in the house mouse, deer mouse and rat^{8,9}, and the different heavy chain immunoglobulin genes are closely linked in man¹⁰, mouse¹¹ and rabbit¹². There are, of course, other linkages which have not been so preserved^{8,9,13}.

The changes which tend to obscure ancestral homologues are Robertsonian fusion, translocations, inversions, gene duplications and deletions, insertions of constitutive heterochromatin composed of repetitious DNA, and rearrangement of nucleolus organizers. There are, however, several factors that favour the retention of recognizable banding patterns between ancient homologues. One of these factors is that extensive rearrangement of the genome can take place by Robertsonian fusion without affecting in any way the banding relationships within the arms. This is also true, to a lesser extent, of translocations and inversions. Furthermore, the extensive mutational evolution that is taking place at the level of base substitutions would have no effect on the banding patterns, and even the occurrence of gene duplications and deficiencies would have only a relatively minor effect on such a gross feature as the banding patterns seen by optical microscopy. In even closely related species the occurrence of marked differences in the type, amount, and placement of satellite DNA^{14,15} may help to produce rather significant karyotypic and banding changes for comparisons between species. These can be minimized by restricting comparisons to the giemsa banding non-centromeric regions.

The correlation between chromosome banding and late replication patterns¹⁶ suggests that the amount of late replicating DNA has also been well preserved over long evolutionary periods. This implies that some functioning and useful genes might be present in this part of the genome, and is consistent with the observation that in many cases the late replicating¹⁷ or heterochromatic¹⁸ DNA is no more highly repetitious than that of euchromatin.

These considerations could also have significant implications for the mapping of human chromosomes. A major problem with studies of humans is that mating cannot be controlled. If linkage groups are as well preserved as these observations suggest, linkage could be studied in any primate (or possibly other mammal) and the observations obtained should, with a certain degree of probability, apply also to humans. For human chromosome abnormalities, it suggests that trisomies or deletions involving ancestral homologues have similar features. Some suggestion of this can be seen in the clinical similarities in the two deletion syndromes 4p- and 5p-¹⁹.

Finally, the base sequence of genes on ancestral pairs of homologues should show greater similarity with each other than with unrelated chromosome sets. This could have some practical application in gene mapping. For example, if the unlinked beta and alpha haemoglobin genes were originally duplicated as a result of this tetraploid event, and evidence were found for the assignment of the beta gene to a certain chromosome, the ancestral homologue of that chromosome would be a logical site to look for the alpha gene. A more immediate example is found with LDH A and B genes. The duplication of these genes probably occurred as the result of ancestral tetraploidy^{1,2}. Ruddle and Chen²⁰ have found that the A locus is on chromosome 11 and the B locus is on 12. As shown in Fig. 1, the banding of these two chromosomes suggests they are ancestral homologues.

If linkage groups are well maintained throughout evolution it is possible that there are certain ideal combinations of interrelating genes which, once maximized, are difficult to break down. Simpler explanations, however, are more likely³. The events which are primarily responsible for the breakup of linkage groups, such as inversions and translocations, tend to affect only one of a pair of homologues at a time. While an animal is in this heterozygous state, crossovers between the rearranged and the normal homologue will result in reduced

fertility by production of di- and nullo- centrics, and duplications and deficiencies. Although a significant amount of rearrangement is seen in related *Drosophila* species, in this organism paracentric inversions do not reduce fertility because in the egg the second division spindles are arranged in tandem and only the terminal nuclei, which do not contain the di- or acentric chromosomes, form the zygote²¹.

These linkage breaking phenomena will thus be strongly selected against, and less disturbing events such as Robertsonian fusions, gene duplication, satellite DNA accumulation, single or multiple gene mutations, and geographical isolation will be the favoured pathways for the development of new species.

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for the independent sequential reactivation of transcription of the major classes of RNA by these nuclei.

By using mouse blastomere-A9 cell hybrids we have shown that the controls of RNA synthesis during early embryological development of mouse are similar to those observed in *Xenopus*.

Two and 4-cell embryos were obtained from Swiss, super-ovulated¹² pregnant mice, 42 and 55.5 h respectively after the second superovulatory injection. The zona pellucida of the isolated embryos was removed with 0.5% pronase in Hanks balanced salt solution before fusion. The embryos were then gently placed on top of a monolayer of A9 cells in a Leighton tube and ultraviolet-inactivated Sendai virus¹³ was added at a final concentration of 200 haemagglutination units (HAU) in the medium usually used for the growth of A9 cells (medium 199 supplemented with 10% calf serum and 0.5% extra glutamine). The blastomere-cell-virus mixture was kept at 4° C for 2 min then incubated at 37° C for 30 min. The heterokaryons and unfused control blastomeres were then isolated and suspended in Mintz medium¹⁴ containing 20 μ Ci of ³H-uridine/ml. (specific activity 19.4 Ci/mmol) and incubated for an additional 2 h at 37° C. Excess (750-fold) unlabelled deoxycytidine was added to the culture medium to prevent the incorporation of ³H-uridine into DNA¹⁵. The heterokaryons and control blastomeres were harvested by a modified orcein squash technique. Slides were frozen on dry ice, the coverslips removed, rinsed thoroughly in 70% ethanol, dipped in a 1 : 3 mixture acetic acid : absolute ethanol for 15 min, and dried. The cells were treated with ice cold 10% TCA for 20 min and autoradiographed ('Kodak liquid NTB3' emulsion; exposure time 96 h). After development of the emulsion the cells were restained with 0.4% azure B in 1% potassium biphthalate, examined under the microscope, photographed, and the concentration of grains over the nuclear area determined.

Table 1 Mean Numbers of Grains per Nuclear Area for a Minimum of 100 Nuclei ($\pm$ s.e.m.)

Developmental stage	Embryo control	Number of embryo nuclei in heterokaryons	Number of A9 nuclei in heterokaryons
2-cell	38.2 $\pm$ 2.9	84.5 $\pm$ 5.3	18.2 $\pm$ 1.1
4-cell	383.8 $\pm$ 36.5	718.0 $\pm$ 32.7	189.7 $\pm$ 9.0
Ratio of 4-cell : 2-cell	10.0 : 1 *	8.5 : 1 *	10.4 : 1 *

* If the 95% confidence limits of the means are calculated, the ratio of 8.5 : 1 may range from a minimum of 6.9 : 1 to a maximum of 10.6 : 1. Similarly, the ratio of 10.4 : 1 may range from 8.4 : 1 to 13.0 : 1. Therefore, within the 95% confidence limits, no two ratios are statistically different from each other.

Control of Nuclear RNA Synthesis in 2-Cell and 4-Cell Mouse Embryos

CHANGING patterns of RNA synthesis during early embryological development have been well established in a number of organisms¹⁻³. In the mouse, very small amounts of only high molecular weight RNA synthesis can be detected at the 2-cell stage⁴. Nucleolar RNA synthesis begins during the 4-cell stage^{5,6} and newly made ribosomal and transfer RNAs can be detected at this stage^{7,8}. The overall increase in RNA synthesis per cell between the 2 and 4-cell stages of mouse development is between five and ten-fold⁴.

Nuclear transplantation in *Xenopus* has led to an understanding of some of the cytoplasmic controls of nuclear RNA synthesis in early development^{9,10}. RNA synthesis by the adult nuclei transplanted into enucleated eggs is at first suppressed⁹ but, as normal development proceeds, the temporal sequence of initiation of informational, transfer, and ribosomal RNA becomes the same as that observed in normally fertilized embryos¹¹. This implies the existence in the egg cytoplasm of factors responsible for the suppression of RNA synthesis in previously competent nuclei, and of other factors responsible

The average number of grains per nuclear area in 2-cell control blastomeres or in 2-cell blastomere/A9 heterokaryons was about one-tenth the average number of grains over the nuclei in 4-cell control blastomeres and 4-cell blastomere/A9 heterokaryons (Table 1; Figs. 1 and 2). Nucleolar RNA synthesis could not be detected in any nucleus in 2-cell cytoplasm, but was evident in both somatic and embryonic nuclei 4-cell material. These results are consistent with the difference in RNA synthesis between 2 and 4-cell blastomeres *in vitro*, treated with neither virus nor pronase⁴. Since both embryonic and somatic nuclei in heterokaryons in culture incorporated ³H-uridine into their RNA, and the patterns of their ³H-uridine incorporation were the same as those observed by other workers⁴, it seems that the heterokaryons remain alive and synthetically active after virus-mediated fusion. The cells examined in this study were true heterokaryons and not artefacts resulting from adsorption of cells on the blastomere surface because (1) when no virus, or very low concentrations of virus, were used, no cells with more than one nucleus were found; (2) a change in the RNA-synthetic activity of all somatic nuclei was observed after fusion with embryos; and (3) there

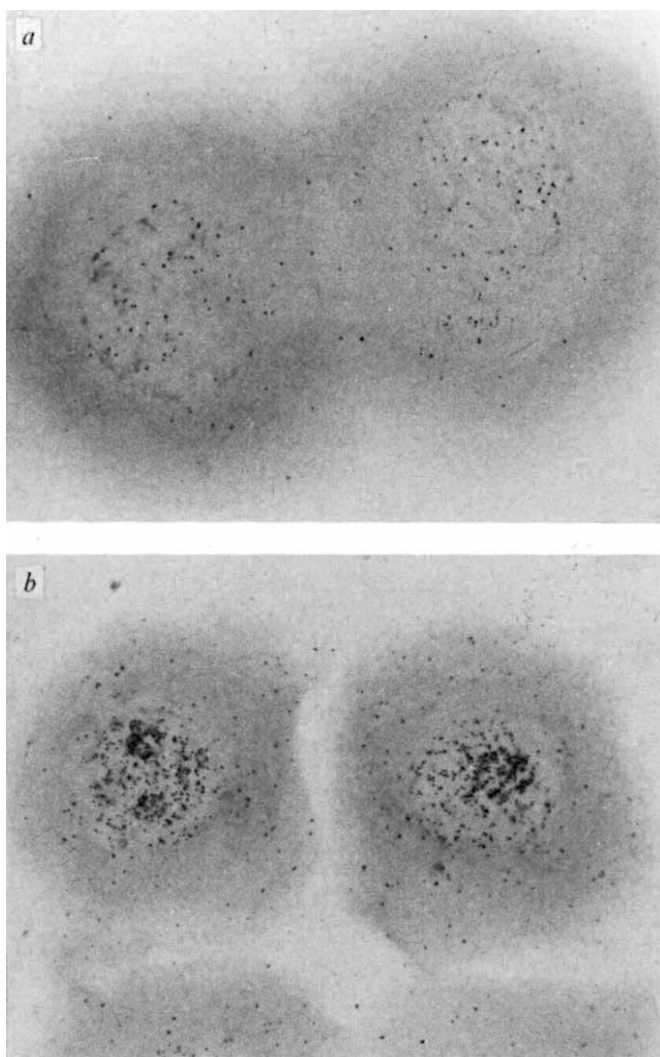


Fig. 1 Autoradiograms of control 2-cell and 4-cell mouse embryos. *a*, Autoradiogram of a 2-cell control embryo incubated for 2 h in a medium containing 20 μ Ci of 3 H-uridine/ml. *b*, Autoradiogram of two blastomeres from a 4-cell control embryo treated in the same way as the 2-cell embryo. Note the difference in 3 H-uridine incorporation between blastomeres from 2-cell and 4-cell embryos. Nucleolar RNA synthesis was not detected in any nucleus of 2-cell blastomeres, but was evident in blastomeres from 4-cell embryos.

was no indication that 3 H-uridine incorporation into somatic nuclei is regulated independently of embryonic cytoplasm, or that 3 H-uridine was incorporated into RNA of somatic nuclei without parallel incorporation into the blastomere nucleus of the same heterokaryon. Evidence that 3 H-uridine was incorporated into DNA-primed RNA comes from two sources. First, the isotopic labelling in all control cells and in all heterokaryons was sensitive to RNAase but not DNAase. Second, the sensitivity to actinomycin-D of incorporation of 3 H-uridine into RNA was dose dependent.

A9 nuclei synthesizing large amounts of RNA before fusion synthesize very little RNA after fusion with 2-cell blastomeres. Four-cell blastomere/A9 heterokaryons under identical conditions incorporated a significant amount of 3 H-uridine, ruling out the possibility of injury caused by the fusion procedure or the inadequate flattening of the heterokaryons before autoradiography. The cytoplasm of 2-cell mouse embryos seems to contain factors capable of suppressing RNA synthesis, and such factors are largely absent from the 4-cell blastomeres. A9 nuclei in heterokaryons are regulated in the same way as embryo nuclei and further extrapolation of the data in Table 1 shows that the addition of A9 nuclei and cytoplasm to 2-cell or 4-cell blastomeres stimulates the embryo

nuclei in heterokaryon to incorporate into RNA approximately twice the normal amount of 3 H-uridine, a further evidence that RNA synthesis in 2 and 4-cell embryos is influenced by cytoplasmic controls.

Table 2 Uptake of 3 H-Uridine into Acid-soluble Fractions of 2 and 4-Cell Embryos

Developmental stage	Number of embryos	Total c.p.m. above background
2-cell	250	329.8
4-cell	150	506.6

Ratio of c.p.m. of 100 4-cell embryos to c.p.m. of 100 2-cell embryos = 1.28 : 1.

The equivalent uptake of 3 H-uridine and its conversion to 3 H-UTP in 2 and 4-cell embryos confirms that very little RNA is synthesized by 2-cell blastomeres and 2-cell/A9 heterokaryons in comparison with 4-cell embryos and 4-cell/A9 heterokaryons. The 2 and 4-cell embryos were incubated with labelled isotope, and were then washed ten

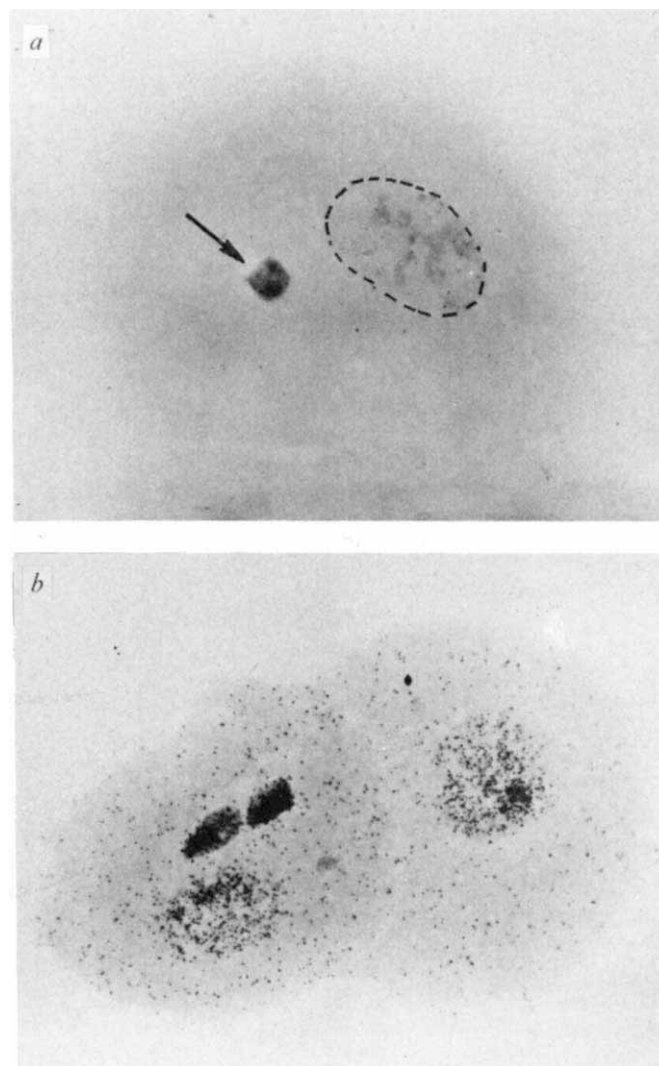


Fig. 2 Autoradiograms of experimental 2-cell and 4-cell mouse embryos. *a*, A 2-cell blastomere/A9 heterokaryon, incubated for 2 h in a medium containing 20 μ Ci of 3 H-uridine/ml. Both A9 (indicated by an arrow) and blastomere nuclei (dotted ring) show very little 3 H-uridine incorporation as in control 2-cell blastomere. *b*, Autoradiogram of two blastomeres from a 4-cell embryo, one of which has been fused with two A9 cells. There are changes in 3 H-uridine incorporation in both A9 and blastomere nuclei.

times with ice cold Mintz medium. The embryos were pipetted into 0.5 ml. of 0.6 M HClO₄ and extracted at 4° C for 20 min and occasionally agitated. The mixture was neutralized with an equal volume of 0.6 M KOH and the KClO₄ precipitate was removed by centrifugation at 500g for 10 min. The supernatant was counted in 10 ml. of Bray's scintillation fluid¹⁷ and quenching was determined by comparisons to an external standard. Table 2 shows that the uptake of ³H-uridine into acid-soluble pools was equivalent in 2 and 4-cell blastomeres. Tasca and Hillman¹⁸ have also shown little change in uptake at these stages.

Daentl and Epstein¹⁹ have shown that 79% of the radioactive acid-soluble fraction of 2-cell embryos is UTP and this proportion was reduced to 50% by the time embryos reached the 8 to 16-cell stage. The intracellular conversion of ³H-uridine to ³H-UTP is not the rate-limiting step in the kinetics of ³H-uridine incorporation into RNA at either the 2 or 4-cell stage. We have confirmed these results using a similar DEAE cellulose chromatographic separation. The final specific activities of the ³H-UTP pools have not yet been estimated for blastomeres at any pre-implantation stage mouse development and the results, therefore, can only be interpreted as incorporation of ³H-uridine into RNA and not as net rate of RNA synthesis. Although the patterns of morphogenesis in mouse and *Xenopus* are very different, their control of nuclear RNA synthesis during early development is similar.

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Inhibition of Peptide Chain Elongation

ABRIN, a protein present in the seeds of *Abrus precatorius* L. (*Leguminosae*), is highly toxic in animals and man, especially when administered parenterally¹⁻³. We show here that abrin is a potent inhibitor of peptide chain elongation although the precise point at which the compound acts has not been determined. Recently we found that ricin, a toxic protein present in the seeds of an entirely different plant (*Ricinus communis* L. (*Euphorbiaceae*)), inhibits protein synthesis in a similar fashion⁴.

Abrin was extracted from *Semen jequiriti* (the seeds of *Abrus precatorius*) with 5% acetic acid and purified according

to Lin *et al.*⁵ with some modifications. The method, which will be described in detail elsewhere, involves fractionated precipitation with ammonium sulphate and dialysis against H₂O followed by chromatography on a 'DEAE-Sephadex-50' column and then on a CM-52 column. The protein obtained in this way migrated as a single band in polyacrylamide gel electrophoresis (Fig. 1a).

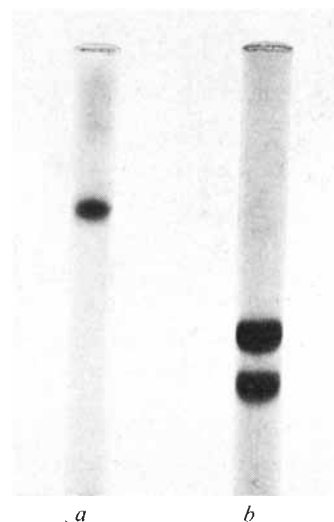


Fig. 1 Polyacrylamide gel electrophoresis of (a) intact and (b) dissociated abrin. a, The intact toxin was submitted to polyacrylamide gel electrophoresis at pH 4.5 in 7% gels⁶ in the absence of urea. b, The toxin was treated with 8 M urea containing 1% mercapto-ethanol and 1% sodium dodecyl sulphate and submitted to polyacrylamide gel electrophoresis in 13% gels⁷ containing 0.1% sodium dodecyl sulphate. To estimate molecular weights, the following standards were used: serum albumin (bovine), ovalbumin (from hen's egg), chymotrypsinogen, and γ -globulin.

The molecular weight estimated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate was found to be 65,000 in accordance with the findings of other authors⁸. When the toxin was treated with mercapto-ethanol before gel electrophoresis, two approximately equal bands appeared (Fig. 1b). The migration rates corresponded to molecular weights of 35,000 and 30,000. These results suggest that the intact toxin is composed of two subunits held together by S-S bonds. Interestingly, ricin appears also to consist of two subunits, held together by S-S bonds and having molecular weights of 35,000 and 30,000 (our unpublished results).

The effect of abrin on protein synthesis was studied in a cell-free system prepared from a rabbit reticulocyte lysate according to Lingrel⁹. The results (Fig. 2) demonstrate that in the absence of toxin incorporation of a labelled amino-acid into protein in this system proceeded almost linearly for 30 min. When 0.1 μ g of abrin was added, the rate of incorporation declined after 5 min and almost stopped after about 10 min. In the presence of 1 μ g of abrin the protein synthesis was strongly inhibited after 5 min. When abrin had been denatured by preincubation at 90° C for 5 min, however, protein synthesis proceeded normally for about 15 min, but then declined. Previous workers have shown that abrin treated in this way has lost 99% of its toxic effect on living animals¹⁰. The inhibition of protein synthesis in the present system after incubation for 15 min might be due to partial renaturation of the toxin during incubation.

The present data agree with those obtained on cells in culture; it was found that abrin inhibited protein synthesis to a higher degree than RNA and DNA synthesis¹¹. Our results suggest that the toxic effect of abrin in animals may be accounted for by its effect on protein synthesis. The fact that

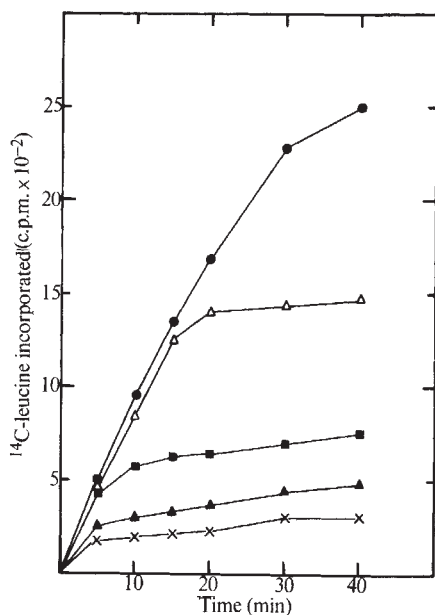


Fig. 2 Effect of abrin on protein synthesis in a lysate from rabbit reticulocytes. One of the tubes was used as control (●); others contained, 0.1 μ g abrin (■), 1 μ g abrin (▲), 2 μ g abrin (×), and 1 μ g abrin preincubated at 90°C for 5 min (Δ). The incubation mixtures contained in a total volume of 0.5 ml.: 0.2 ml. lysate, 10 mM tris-HCl (pH 7.4), 100 mM ammonium acetate, 2 mM magnesium acetate, 1 mM ATP, 0.2 mM GTP, 15 mM creatine phosphate, 15 μ g/ml. creatine phosphokinase and amino-acids in concentrations varying from 0.01 mM to 0.1 mM and 1.25 μ Ci of 14 C-leucine (uniformly labelled, spec. act. 331 Ci/mol). The incubation was carried out at 28°C. At the times indicated 20 μ l. samples were removed and poured into 1 ml. of 0.1 M KOH and incubated at room temperature for 60 min. Trichloroacetic acid was then added to a final concentration of 10% (w/v) and the haem was converted into a colourless compound by the addition of 1% H_2O_2 . The precipitated protein was then collected on Gelman glass fibre filters type A, incubated with Soluene to solubilize the protein and the radioactivity was counted in a 'Beckman LS-130' scintillation system as described elsewhere⁶.

toxic symptoms do not appear until after some time (one or a few hours) even when several times the lethal dose of the toxin is administered^{1,2} is consistent with this interpretation.

To elucidate the mechanism of inhibition of protein synthesis by abrin, the cell-free system was incubated for 15 min both in the absence and presence of abrin and was subsequently submitted to sucrose gradient centrifugation. In the absence of abrin the absorbance at 260 nm revealed (Fig. 3a) that polysomes were the major component, although some monoribosomes were also found. Most of the trichloroacetic acid-insoluble radioactivity (about 80%) was present on top of the gradient, showing that a considerable number of globin chains had been completed during the incubation. Part of the radioactivity (about 20%) was recovered from the polysome region, indicating the formation of nascent peptide chains. After incubation in the presence of 1 μ g abrin (Fig. 3b) the total radioactivity precipitated by trichloroacetic acid was much lower and, significantly, a much larger fraction of the radioactivity (about 2/3) was now present in the polysome region with only 1/3 on top of the gradient. The results indicate that in the presence of abrin the completion and release of globin chains from the polysomes were strongly reduced. The fact that in the presence of 1 μ g abrin the amount of radioactivity incorporated after 15 min was about 25% of that found in the control is in agreement with the data shown in Fig. 2.

When incubation was carried out in the presence of aurin tricarboxylic acid (ATA) which is known to inhibit the initiation of new peptide chains^{12,13}, as expected, only monoribosomes were observed (Fig. 3c). In this case all the acid-

precipitable radioactivity was found on top of the gradient, demonstrating that the peptide chains initiated prior to the addition of ATA were completed and released during the incubation. When both ATA and abrin were present, however, the polysome structure was partly preserved, and almost half of the total radioactivity was now found in the polysome region, indicating that abrin had prevented the "run off" of the ribosomes and inhibited the completion and liberation of the already initiated peptide chains.

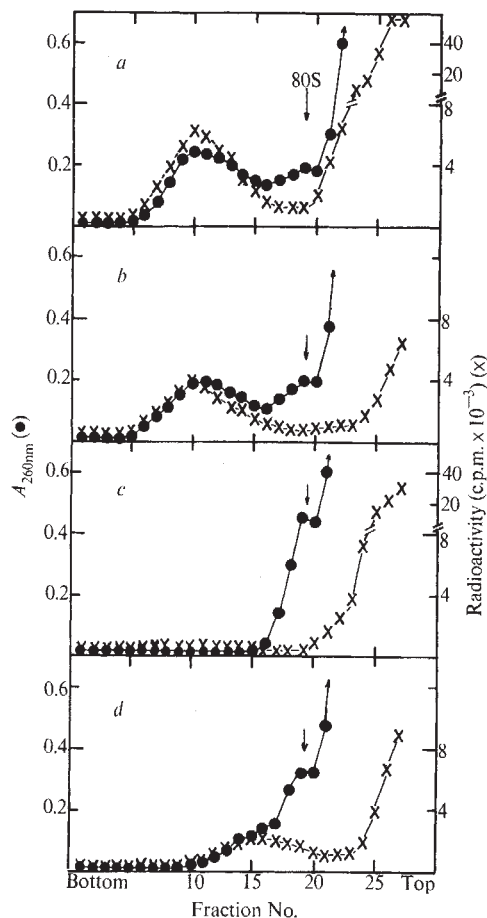


Fig. 3 Effect of abrin and ATA on the polysome pattern and nascent peptide chains in a lysate from rabbit reticulocytes. The cell-free system (prepared as in Fig. 2) was divided into four; one sample (a) was used as control whereas to the others were added: (b) 1 μ g abrin, (c) 5×10^{-5} M ATA and (d) 5×10^{-5} M ATA + 1 μ g abrin. Then 3 μ Ci of 14 C-leucine was added and the tubes were incubated at 28°C for 15 min. After incubation 300 μ l. aliquots from each tube were layered on to 0.5–1.0 M sucrose gradients made up in 0.15 M KCl containing 1 mM MgCl_2 and 10 mM triethanolamine (pH 7.5) and centrifuged in rotor 'SW-50.1' at 130,000g for 60 min. Fractions were collected by puncturing the bottom of the tubes and after addition of 0.8 ml. of H_2O the absorbance at 260 nm and the acid-precipitable radioactivity were determined.

The present results are very similar to our recent findings with ricin⁴. It is interesting that both toxins require a few minutes to manifest their effects in the cell-free system. Since they do not appear to affect the charging of tRNA (unpublished data), it is possible that the toxic proteins act by gradually inactivating some component essential for peptide chain elongation.

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Sucrose Induction of Hepatic Hyperplasia in the Rat

SUCROSE in the diet¹ of rats leads to production of livers heavier than those of rats fed diets with starch². We report here experiments to determine whether the increase in liver size induced by sucrose is due to an increase in cell size (hypertrophy) or to an increase in cell number (hyperplasia) or to both.

Male Sprague-Dawley rats were fed on a stock laboratory diet from the age of 4 weeks, until they weighed 200–230 g at 60–70 days old. They were then placed onto the purified diets described below. Water and food were given freely during the course of the experiments. At the end of the experiment the rats were killed, the livers were rapidly removed, rinsed in ice-cold saline, dried on filter paper, and weighed. One gram of the liver was homogenized with 9 ml. 0.15 M NaCl in 0.2 M Tris buffer, pH 9.6, at 0° C.

DNA was determined by the method of Volkin and Cohn³. Trichloroacetic acid was added and the amount of true protein in the precipitate produced was determined by the micro-Kjeldahl method.

Results are reported as significant when $P < 0.05$, as calculated by the Mann-Whitney⁴ method.

In the first experiment five groups, each of six rats, were put on diets in which the carbohydrate was corn starch, sucrose, fructose, glucose or maltose. The composition of the diets was carbohydrate 70%, casein 24%, arachis oil 0.8%, salts 4%, 'solka-floc' cellulose 2% and vitamin mixture⁵. After 30 days on these diets, the animals were killed and the livers examined.

Table 1 Effect of Dietary Carbohydrates on Rat Liver. Median Values, 6 Rats in Each Group

Dietary carbohydrate	Liver weight g	Liver weight g/100 g body weight	DNA mg/g liver	DNA mg/g protein	Cell No. $\times 10^9$	Cell mass, pg
Starch	11.1	3.5	2.58	17.2	4.4	2.41
Sucrose	13.6*†	3.8*†	2.63	17.5	6.3*†	2.36
Fructose	15.3*†	4.3*†	2.13*†	15.7*	5.2*†	2.91*†
Glucose	11.4	3.3	2.41	16.4	4.4	2.56
Maltose	12.4	3.4	2.49	15.7	4.8	2.44

* Differs significantly from starch.

† Differs significantly from glucose.

The livers of rats fed on diets with starch, glucose or maltose showed no significant differences in weight or DNA content (Table 1). Sucrose and fructose produced heavier livers and a greater total DNA content than did starch, glucose or maltose. With sucrose, there was no difference in DNA content when it

was calculated in terms of unit liver weight or protein, indicating that there was an increase of about 40% in cell number, but no change in cell size.

With fructose, there was an increase in total DNA, but a decrease in terms of unit liver weight. This suggests that fructose produced an increase both in cell number and in cell size.

In the second experiment, eight groups, each of seven rats, were given diets with casein contents of 7%, 10%, 15% or 20%, and either starch or sucrose as the major carbohydrate. Diets were made up as in the first experiment, except for the proportion of carbohydrate and casein. The sucrose diets were 70% sucrose, the remainder being made up with starch. The starch diets contained 72–85% starch to compensate for the reducing protein level. After 90 days on these diets the rats were killed.

Diets with the higher amounts of protein gave the same concentration of DNA in terms of unit liver weight or unit liver protein with either sucrose or starch (Table 2). Since, however, sucrose produced significantly heavier livers than did starch, total liver DNA was increased by sucrose. This increase was significant with 10% and 20% protein but was not significant with 15% protein, possibly because there was an unusually high variation in the results.

Table 2 Effects of Starch and Sucrose Diets on Livers of Rats Fed Different Levels of Protein. Median Values, 7 Rats per Group

Dietary protein level	Dietary carbohydrate	Liver weight g	Liver weight g/100 g body weight	DNA mg/g liver	DNA mg/g true protein	Cell No. $\times 10^9$	Cell mass pg
7%	Starch	9.3	3.1	3.5	22.6	5.0	1.8
	Sucrose	8.1	3.7†	3.6	23.2	4.8	1.7
10%	Starch	10.3	3.0	3.5	21.9	6.1	1.7
	Sucrose	12.4*	3.6†	3.3	21.8	6.8†	1.8
15%	Starch	12.3	2.9	3.4	20.7	7.0	1.8
	Sucrose	13.4	3.5†	3.3	20.4	7.4	1.8
20%	Starch	13.7	3.2	3.4	19.8	7.4	1.8
	Sucrose	14.6*	3.7†	3.3	20.6	8.0*	1.8

* $P < 0.05$.

† $P < 0.001$.

A diet with sucrose and 7% protein resulted in a significant reduction in weight gain of the rats. This accounts for the fact that, although sucrose produced livers that weighed less in absolute terms, they were heavier when expressed per 100 g body weight. Similarly, the total DNA and the total number of cells did not differ in livers of rats fed either sucrose or starch, but when expressed in terms of body weight the livers of the sucrose-fed rats had more DNA and thus a greater number of cells. Total liver DNA per 100 g body weight was 133 mg with sucrose and 108 mg with starch.

Calculation of the number and size of the cells is based on the assumption that the DNA content of the nucleus is constant. If it is also assumed that all rat tissues are made up of cells with diploid nuclei, then the weight of DNA in the average cell⁶ is 6.2 pg. The hepatic cells of the rat, however, are known to contain polyploid cells, and there is an increase in binucleated cells with age^{6,7}; calculations based on the figure of 6.2 pg will thus not give correct absolute values. We were, however, less interested in the absolute number or size of cells than in the general effect on them of dietary change. We have established that the increase of liver size induced by the dietary substitution of sucrose for starch is caused by an increase in the number of cells, or at least in the number of nuclei. With fructose instead of starch, the effect is due to an increase in both cell number and cell size.

The rats in our experiment were aged 60–70 days when the experimental purified diets were introduced. It has been found that in rats fed a stock diet growth of the liver after the age of about 44 days takes place by increase in cell size only⁸.

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Phenylalanine Metabolism altered by Dietary Dieldrin

CHANGES in the metabolism of phenylalanine have been related to altered homeostasis of brain enzymes and mental deficiency in mammals¹. Little is known, however, about the situation in fish, and we wish to describe the chronic effects of the pesticide dieldrin on these metabolic pathways in the

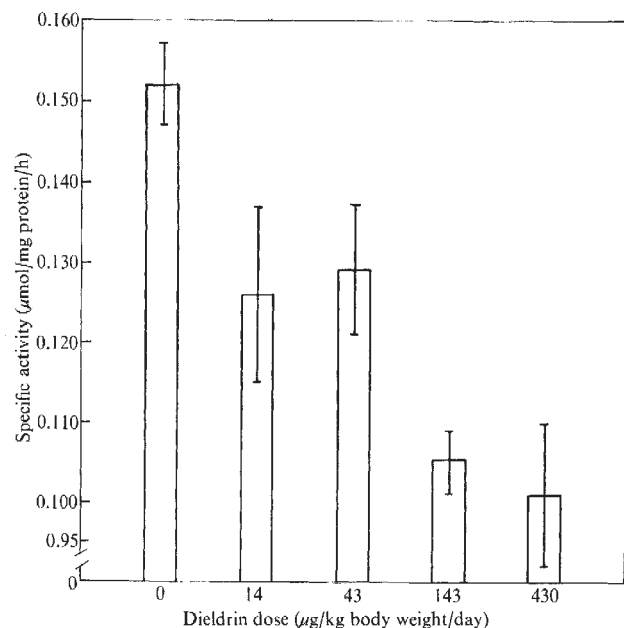


Fig. 1 Liver phenylalanine hydroxylase activity as affected by dietary dieldrin. $\bar{X} \pm s_x$ is presented for each group, where $n=12, 8, 10, 7$, and 7 for the $0, 14, 43, 143$, and $430 \mu\text{g/kg}$ groups, respectively. All groups receiving dieldrin had significantly lower activity relative to the control group ($\text{LSD}_{0.05}$, F value 7.20). Enzyme activity expressed in $\mu\text{mol/mg tissue/h}$ showed similar trends among dosage groups as the specific activity.

rainbow trout (*Salmo gairdneri*). According to the National Pesticide Monitoring Program², this is one of the most prevalent pesticides in the aquatic environment, having been found in 93% of fish samples from across the United States in concentrations of 0.01 to $1.6 \mu\text{g/g}$ (p.p.m.).

Mehrle³ has already found that in rainbow trout fed $3.6 \text{ mg dieldrin/kg}$ of food for 160 days, phenylalanine increased approximately 50% and the concentrations of ten other amino-acids were altered. Changes in plasma amino-acid concentrations have been observed in people occupationally exposed to pesticides^{4,5}. Dieldrin fed to rainbow trout for 240 days affected the activities of the brain enzymes glutamine transferase, glutamate dehydrogenase, glutamate-pyruvate transaminase, and glutamate-oxaloacetate transaminase, and the concentration of nine brain amino-acids⁶. Electron microscopy of liver tissue suggested that mitochondria and endoplasmic reticulum were also altered. We have now investigated the effects of dietary dieldrin on liver phenylalanine hydroxylase activity, serum phenylalanine, and phenylpyruvic acid concentrations in urine.

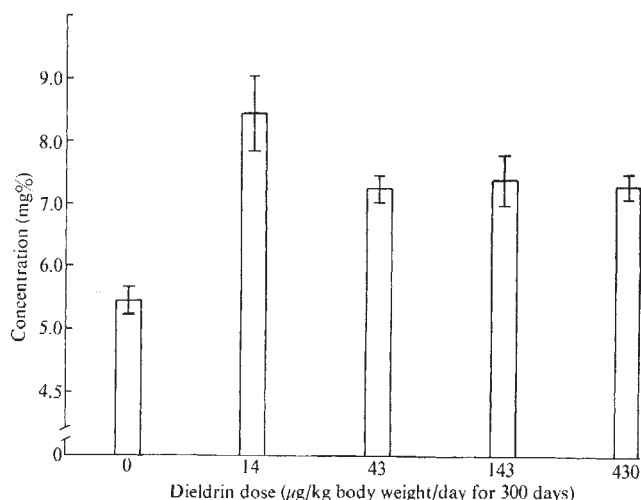


Fig. 2 Changes in blood phenylalanine levels after dieldrin exposure. $\bar{X} \pm s_x$ is presented for each group ($n=8$). All groups receiving dieldrin had significantly higher concentrations relative to the control group ($\text{LSD}_{0.05}$, F value 9.97).

Rainbow trout weighing approximately 1.7 g were placed into each of five 570 l . fibreglass tanks supplied with 5 l./min of 17°C well water. Dieldrin (1,2,3,4,10,10-hexachloro-6-7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene, 99% plus, Shell Oil Co.) was incorporated into the diet to give concentrations of $0, 0.36, 1.08, 3.6$, and $10.8 \mu\text{g/g}$ of food, corresponding to doses of $0, 14, 43, 143$, and $430 \mu\text{g dieldrin/kg body weight/day}$. The fish were fed daily portions equivalent to 4% of their body weight for 300 days after which, liver, blood and urine samples were analysed. Four fish from each group were also analysed for whole body residues of dieldrin. Each group was weighed monthly and feeding rates were adjusted accordingly during exposure.

Liver phenylalanine hydroxylase activity was measured according to the method of Freedland *et al.*⁷ as modified by Voss and Waisman⁸. The reproducibility of the method on six replicate samples was 1.2%. Serum phenylalanine was estimated fluorimetrically⁹ as adapted to fish serum. Reproducibility on eight replicate samples was 2.0% and accuracy was 99%. Values derived from the fluorimetric method are comparable with those determined by gas-liquid chromatography³. Phenylpyruvic acid was determined by a modification of the method by Schepartz¹⁰ which is specific for the aryl pyruvates, phenyl-, *p*-hydroxyphenyl-, indole-, and imidazole-pyruvic acids. Urine ($200 \mu\text{l}$.) was added to $200 \mu\text{l}$. of phenazine methosulphate (2 mg/ml . of 0.75 M potassium

phosphate buffer, pH 7) and the colour development was measured at 675 nm after 1 h. Reproducibility of eight replicate samples was 1.8% and accuracy was 97.5%. Phenylpyruvic acid gave twice the response of that produced by *p*-hydroxyphenylpyruvic and imidazolepyruvic acids, whereas indolepyruvic, indolelactic, phenyllactic, phenylacetic, *p*-hydroxyphenylacetic and *p*-hydroxyphenyllactic acids produced negligible colour in our reaction conditions.

Growth rates were not altered during 300 days of exposure to dieldrin. Wholebody dieldrin residues were 0.41, 0.79, 2.10, and 6.23 µg/g (p.p.m. wet weight) in the 14, 43, 143, and 430 µg/kg groups, respectively, whereas no dieldrin was detected in the control group. Residues in fish resulting from exposure to the three smallest doses were in the range of those reported by the National Pesticide Monitoring Program².

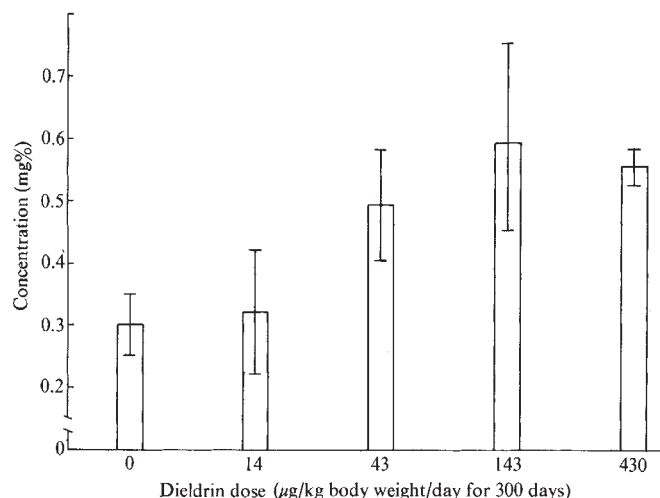


Fig. 3 Effects of dietary dieldrin on the concentration of urine phenylpyruvic acid. $\bar{X} \pm s.e.$ is presented for each group, where $n=12, 8, 8, 6$, and 8 for the $0, 14, 43, 143$, and 430 µg/kg group, respectively. Groups receiving the highest three dosages had significantly greater concentrations than the control group ($LSD_{0.05}, F$ value 3.90).

Liver phenylalanine hydroxylase activity was significantly decreased ($P<0.05$) by all doses of dieldrin (Fig. 1). There was a negative correlation between dieldrin dose and enzyme activity ($r = -0.888, P<0.01$). *In vitro* studies have shown that concentrations of 0.4, 4.0, and 40 mg/l. (p.p.m.) of dieldrin in the enzyme incubation medium do not alter phenylalanine hydroxylase activity. This suggests that the decreased enzyme activity after dietary exposure was due to inhibition by feedback from other metabolic pathways. In addition, dieldrin's effect on phenylalanine hydroxylase activity seems to be persistent, for the liver enzyme activities in the groups given the largest and smallest doses were still significantly ($P<0.05$) lower than the control group after the fish were maintained on control diets (0 dose) for 4 months after the initial 300-day exposure to dieldrin. Macek *et al.*¹¹ reported the half-life of dieldrin to be 44 days in rainbow trout. Thus, after approximately three half-lives, the enzyme activity was still decreased.

Serum phenylalanine concentration was increased by all doses of dieldrin (Fig. 2). The concentration in the group given to the smallest dose increased by 55%, whereas in the groups receiving the three largest doses it increased by approximately 34%. Thus, a mild hyperphenylalaninaemia was induced by chronic dietary dieldrin. A similar increase has been observed before³.

The concentration of urinary phenylpyruvic acid, a phenylketo-acid metabolite of phenylalanine, increased in the groups receiving the three largest doses of dieldrin (Fig. 3). In the group receiving 43 µg/kg it increased 63%, whereas in the groups receiving the next largest doses it increased 97% and

83%. There was a significant correlation between decreased phenylalanine hydroxylase activity and increased urine phenylpyruvic acid concentrations among groups receiving different doses ($r=0.860, P<0.01$).

The biochemical properties of phenylalanine hydroxylase have been studied by several investigators^{12,13}. The cofactors NADP and dihydrobiopterin are required for the enzymatic conversion which is actually effected by two enzymes—phenylalanine hydroxylase and dihydropteridine reductase. We cannot ascertain from our data the mode by which dieldrin altered enzymatic activity. Since dieldrin did not alter *in vitro* activity we assume that metabolic products from other pathways affected by dieldrin could alter the phenylalanine hydroxylase complex. Preliminary results suggest that phenylalanine hydroxylase activity is also significantly decreased in rats chronically exposed to dietary dieldrin. Thus the effect of dieldrin on this enzyme system is not specific for fish—mammalian systems respond in a similar manner.

An inherited defect in phenylalanine metabolism, phenylketonuria, is characterized by inhibition of phenylalanine hydroxylase, increased blood phenylalanine, increased urinary keto-acids, and mental deficiency. Our study has shown that dieldrin has a marked effect on phenylalanine metabolism and can induce the biochemical manifestations of phenylketonuria; however, the effects of dieldrin on learning ability in fish remain to be elucidated. We suggest that results from this study are indicative of subtle, chronic effects of dieldrin that may alter the adaptability and survival of rainbow trout. Further research is also warranted in mammals since preliminary results suggest that dieldrin decreased phenylalanine hydroxylase activity in rats.

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Attack Behaviour in Mice inhibited by Δ -9-Tetrahydrocannabinol

MARIJUANA and its active major ingredient, Δ -9-tetrahydrocannabinol (THC), reduce fighting among previously isolated mice¹ and decrease preference for the side of a T-maze where they are rewarded by the opportunity to attack². The degree

of suppression of fighting behaviour seemed to be related to the cannabis resin content of the extract administered¹. The behavioural effects which we reported² could not be assessed for a THC dose-dependent relationship as some mice in the low-dosage group received faulty intravenous injections.

We have now investigated the effects of various doses of THC on attack behaviour in mice, using an experimental design that also assessed effects on a learned position response. To determine non-specific effects of the drug, we measured spontaneous activity. We also measured whole brain amines, for correlations have been reported³⁻⁷ between brain amines and aggressive behaviour.

Thirty-six Balb/cJ (aggressive) and fifteen C57BL/6J (passive) mice (Jackson Laboratories, Bar Harbor, Maine) were housed, pre-trained, then trained to a position habit in a T-maze as described previously⁸, except that visual cues were omitted and four daily training trials were given. During 12 days of pre-training a passive mouse was presented to an aggressor for 2 min and the trial was terminated after an attack had been initiated and maintained for 5 s.

Sixteen days of maze training followed, during which the aggressive mice were given four daily trials in the T-maze; one free and one forced choice run comprised each trial. To facilitate acquisition⁴, the aggressor had an opportunity to attack a passive mouse in the start box section before the initial run. Runs to the side designated as correct, counter-balanced over groups, were rewarded by allowing 10 s for the aggressor to attack and fight a passive mouse in the goal section. Runs to the incorrect side resulted in confinement to the goal box for 15 s. Mice were run in groups of four, each mouse completing trial one before trial two and so on. This required 15 to 20 min per group. Passive mice were used in turn so that they seldom encountered the same aggressive mouse.

During T-maze training, we recorded latency of attack in the start box section, number of free choices to the correct side, and the time required to complete a run. Fights initiated by mice on correct free-choice runs were tabulated after day 4.

After 16 days of T-maze training, nine mice were assigned randomly to each THC level (0.0, 0.6, 1.25 and 2.5 mg/kg). THC in a polyvinylpyrrolidone K-30 vehicle⁹, prepared 24 h before use, was injected into the tail vein at 1 ml/kg volume 30 min before the animal's maze performance. This time delay was based on indications¹⁰ that brain levels of noradrenaline and serotonin may increase 30 to 50 min after injection of THC, for example, the period the mice would spend in the maze.

Post-treatment maze performance was measured on days 18 and 19. Activity for a 3 min period in a photocell apparatus was recorded 30 min after readministration of THC one week after the initial administration. The subjects were then decapitated, and the brains were removed for fluorometric determination¹¹ of whole brain serotonin, noradrenaline and dopamine.

Data were analysed, using the *F* test for group comparisons and the Newman-Keuls method for individual comparisons¹².

Table 1 Mean and Standard Deviation for Number of Correct Choices, Percentage of Attacks, and Attack Latency for Thirty-six Mice on Final Training Day (Day 16) and 30 Min after THC Administration on the Following Day

	THC (mg/kg)	Correct choices	% attacks	Attack latency (s)
Day 16	0.00	3.55 (0.52)	89.89 (16.70)	1.50 (1.66)
	0.60	3.55 (0.52)	74.11 (23.40)	1.50 (0.72)
	1.25	3.22 (0.97)	89.78 (22.80)	1.66 (1.11)
	2.50	3.22 (0.66)	81.56 (18.90)	2.00 (2.34)
Test day	0.00	3.33 (0.86)	67.55 (40.50)	11.88 (21.60)
	0.60	3.55 (0.72)	46.22 (43.90)	20.44 (26.00)
	1.25	2.88 (1.05)	09.22 (18.90)	73.66 (48.50)
	2.50	2.22 (1.39)	02.77 (08.30)	71.22 (47.80)

THC was not administered on day 16. Group designations were made for statistical comparison after testing.

Table 2 Mean and Standard Deviation of Whole Brain Noradrenaline, Serotonin, and DA 33 Min after THC Administration for Thirty-six Mice

THC (mg/kg)	Noradrenaline (µg/g)	Serotonin (µg/g)	Dopamine (µg/g)
0.00	0.401 (0.069)	0.426 (0.074)	0.831 (0.431)
0.60	0.441 (0.113)	0.408 (0.143)	0.719 (0.127)
1.25	0.422 (0.120)	0.501 (0.010)	0.783 (0.360)
2.50	0.470 (0.088)	0.468 (0.073)	0.946 (0.207)

The number of correct choices on the free choice run, analysed in blocks of 3 days beginning with day 5 of T-maze training, increased significantly ($\bar{X}$ = 2.86, days 5-7; 3.15, days 8-10; 3.32, days 11-13; 3.44, days 14-16; $P < 0.01$), which indicated that the opportunity to attack was sufficiently reinforcing to enable the animals to learn the task. Aggressive behaviour, measured by the percentage of attacks when a correct choice had been made, remained stable ($\bar{X}$ = 67%, days 5-7; 75%, days 8-10; 80%, days 11-13; 78%, days 14-16; $P > 0.05$). Latency of attack in the start box also remained stable ($\bar{X}$ = 2.8 s, day 5; 1.6, day 16; $P > 0.05$).

Analysis of the pre-test day data did not reflect significant differences among the groups for any of the measures ($P > 0.05$). On the test day the number of correct choices was not affected significantly by administration of THC ($P > 0.05$). The percentage of attacks on free-choice runs, however, in which the fight-rewarded side of the maze had been chosen was decreased significantly ($P < 0.01$), and the latency of the start box attack was increased significantly as well ($P < 0.01$) (Table 1).

Spontaneous activity, as measured in a photocell apparatus, indicated no significant change due to administration of THC ($\bar{X}$ = 104 (0.0 group), 113 (0.6 group), 111 (1.25 group), and 86 (2.5 group); $P > 0.05$). Similarly, the drug had no effect on whole brain amine levels ($P > 0.05$) (Table 2).

These data indicated that acute administration of THC reduced aggressive behaviour in mice, insofar as it increased attack latency in the start box and reduced attack incidence after an animal chose the fight-rewarded side of the T-maze. The data are congruent with the report¹ that marijuana extract inhibited isolation-induced aggression, and they suggest that the inhibition is caused by the THC component of marijuana. The relation between the amount of THC and inhibition of aggression seems to be positive.

The striking inhibition of attack behaviour in the 1.25 group, in the absence of motor effects, may represent a more or less specific influence of THC on the biochemical and/or physiological systems that underlie aggressive behaviour. During T-maze training, the animals' percentage of attack, a measure of aggressive motivation, remained stable. At the same time, their choice of the fight-rewarded side of the maze, a discrimination measure, increased significantly. As changes in motivation are not reflected necessarily by changes in discrimination performance¹³, we suggest that the principal effect of THC was to inhibit biological systems underlying the animals' motivation for aggressive behaviour. The mechanism of this inhibition is unclear, however. Individual comparisons of the test day data showed that the 0.0 and 0.6 groups made significantly more attacks following a free-choice run to the correct side and that their start box attack latency was significantly lower than that of the 1.25 and 2.5 groups ($P < 0.05$). Analyses of these data for 2 days after THC administration failed to yield significant differences among the groups.

On the day before testing, the mice failed to attack 16% of the time, after selection of the fight-rewarded side. On the test day, this increased to 32% for the 0.0 group, and to 54, 91 and 97% for the 0.6, 1.25 and 2.5 groups, respectively. The nonsignificant decrease in attack behaviour and increase in latency of the start box attack ($P > 0.05$) seen in the 0.0 group from pre-test to test day presumably arose from the handling and injection procedure, which may have disrupted the animals' motivation for aggressive behaviour.

Analyses of the mean running time for free and forced choice runs, respectively, indicated a significant increase ($P < 0.01$) in the time required to run the maze after administration of THC. Individual comparisons of these data showed, however, that only the longer running time of the 2.5 group was significant ($P < 0.05$). The increased running time of this group may represent a debilitating effect of THC on motor behaviour in the maze. The 1.25 group, which also showed significant reductions of aggressive behaviours, required no longer to traverse the maze than did the 0.0 group, which suggests a specific inhibitory effect of THC on aggressive behaviour.

Our data are not congruent with studies³⁻⁷ which showed changes in brain amine levels coinciding with changes in levels of aggressive behaviour. As we have shown in rats, however, 1.25 mg/kg of THC¹⁴ increased levels of serotonin in mid-brain and medulla. Therefore, analyses of amines for brain sections in mice may reveal differential changes that correlate with diminished aggression.

Alternatively, for one species of rodent, aggression has been shown to be cholinergically mediated in hypothalamic structures¹⁵. As cannabis resins¹⁶ Δ -8-THC¹⁷ and Δ -9-THC¹⁸ possess anticholinergic properties, this action may underlie the diminished aggressive behaviour we found.

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Non-Random Segregation during Mammalian Oogenesis

INVESTIGATIONS of the breeding performance and early embryogenesis of the XO female mouse^{1,2} by Cattana³ and Morris⁴ were difficult to explain because the number of XO females

born to XO mothers was considerably less than predicted. Cattana³ suggested there was either a preferential loss of the chromosome sets lacking an X to the polar bodies during meiosis, or death during early embryonic development. Morris⁴ and others^{5,6} concluded that YO was inviable. The proportion of oocytes with and without an X chromosome was determined at metaphase II. The sex-linked Tabby gene was used as a marker in the XO mice, which were of similar stock to those used by Cattana³ and Morris⁴. XO females in succeeding generations were mated to +/y and Ta/y males. Ta/O females were superovulated and autopsied 16–20 h after the human chorionic gonadotrophin (HCG) injection. The freshly ovulated oocytes were liberated from the ampullae and ovarian oocytes from the larger intact follicles. Oocytes at the germinal vesicle stage were cultured for 18–24 h in modified Krebs-Ringer bicarbonate medium⁷, after which most reached metaphase II. The metaphase II cells were placed in 1% sodium citrate solution for 10–20 min. Air-dried preparations were made by the method described by Tarkowski⁸ and stained with 2% Giemsa at pH 7.0 for 45 min. The slides were washed briefly with distilled water, allowed to dry and then dehydrated with xylol and mounted with 'Clearmount'.

Control female heterozygous Ta/+ mice were superovulated and autopsied 17–19 h after HCG. The oocytes were liberated and treated as described above.

The 145 preparations from XO mice and the 105 from the control mice were divided into three groups according to the source of the oocytes. (A) Ovulated oocytes removed from the ampullae. (B) Ovarian oocytes at metaphase II obtained by follicular puncture. (C) Germinal vesicle oocytes obtained by follicular puncture and cultured to metaphase II.

Table 1 shows the numbers of chromosomes present in each group. χ^2 analyses of numbers of oocytes with 19 or 20 chromosomes present in XO and control groups were carried out. Groups A and C showed significant differences ($\chi^2_{(1)} = 11.81$, $P < 0.001$; and $\chi^2_{(1)} = 8.14$, $P < 0.005$ respectively); B showed a trend in the same direction but not a significant difference. When the XO series was compared with a random distribution, A alone was significantly different ($P < 0.02$ —Fisher's exact test). The trend in groups B and C, although not statistically significant because of the small sample size, was also in the same direction. All three groups showed approximately twice as many oocytes with 20 chromosomes (X-bearing gametes) as those with 19 chromosomes (gametes without an X) in the XO series.

In XO female mice there is an unusually low (about 30%) segregation of gametes without an X chromosome in ovulated oocytes. This gives a considerably higher incidence of XX and XY than XO and YO offspring (approximately 2 : 2 : 1 : 1). This segregation gives ratios XO/XX = 0.5, and XO + XX/XY = 1.5. If YO leads to anomalous development then 1/6 or 16.7% of cleavage embryos should be grossly abnormal and inviable. (A YO karyotype has been seen at the first cleavage division (M. H. K., unpublished observations). Morris⁴ found XO/XX = 0.399 at weaning and XO + XX/XX = 1.45 at birth and 1.505 at weaning. He also described two types of abnormality in preimplantation 3.5 day embryos. One group

Table 1 Chromosome Counts from Metaphase II Preparations from XO Female Mice (Ta/O)

Group	No. of metaphase II preparations examined	Chromosomes "clumped"*	Chromosome counts				
			<16	17	18	19	20
A, Ovulated oocytes	90	24	3	1	2	19	41
B, Fresh ovarian oocytes	39	15	2	—	1	5	16
C, Cultured ovarian oocytes	16	1	1	—	1	5	8
Control group	105	34	3	—	3	4	61

* Preparations with inadequate separation of chromosomes.

occurred with equal incidence in the XO and XX litters, and probably accounted for the spontaneous level of preimplantation loss. The second group accounted for only 2% of the embryos from XX litters and 20% in the XO litters. If this group accounted for the whole class of YO zygotes and included a small loss of XO zygotes, the XO/XX ratio would increase from 0.399 at weaning to nearer the postulated 0.5 ratio occurring at fertilization. Cattana³ suggested that non-random segregation of the chromosomes could account for the observations of the XO mouse.

Although there was no previous evidence in the Mammalia non-random segregation had been described in plants⁹. Rhoades¹⁰ found that in *Zea* heterozygous for a terminal heterochromatic knob the homologue with the knob reached the functional embryo-sac in 70% of meioses. If the migration of the chromosome pair on the meiotic spindle had been random 50% would be expected. In flowering plants, meiotic preferential distribution of the B-chromosome occurs. These chromosomes appear as univalents and do not divide at anaphase but distribute themselves towards a privileged pole of accumulation. The male nucleus that receives the B-chromosome has the greater chance of fertilizing the egg¹¹. Seiller¹² found that the behaviour of the X chromosome in *Talaeporia tubulosa* was strongly influenced by the temperature level. With normal and subnormal temperatures the X most frequently passed into the polar body, while under high temperatures and in "over-ripe" eggs the X more often remained in the egg.

The XO mouse results seem to be unrelated to the phenomenon of "affinity"¹³ in which a linkage-like association between centromeres of different chromosomes results in their non-random segregation at meiosis causing chromosomes of the same ancestral origin to tend to travel to the same pole. The closeness of results to previously published breeding data suggests non-random segregation at the first meiotic division.

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Heterogeneous Population of Mitochondria in Higher Plant Cells

It is generally accepted that aspartate is synthesized from oxaloacetate which is generated in the tricarboxylic acid cycle (TCA cycle) operating in the mitochondria. Work with plant tissue culture cells¹, however, showed that, although ¹⁴C-labelled metabolites were oxidized in the TCA cycle, the ¹⁴C-oxaloacetate generated was not available for the synthesis of ¹⁴C-aspartate. This was attributed to the possible presence of more than one type of mitochondria. The concept of a heterogeneous population of mitochondria in plant cells was proposed in 1960 by Avers and King, who used histochemical

techniques to study the mitochondria in the root tips of four species of grass². Wilson and Bonner³ identified two enzymatically different populations of mitochondria isolated from peanuts collected at different stages of germination. Similar

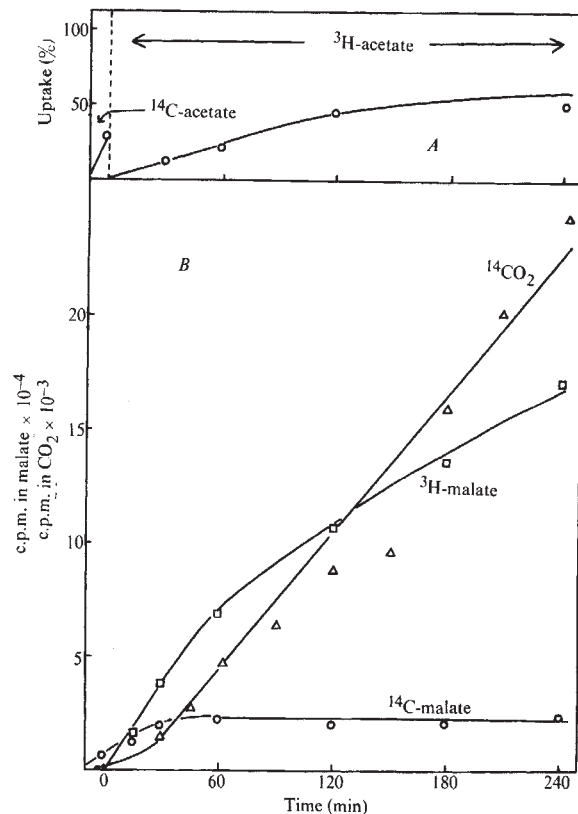


Fig. 1 A, Uptake of ¹⁴C-acetate and ³H-acetate from medium; B, incorporation of ¹⁴C and ³H into malate and CO₂.

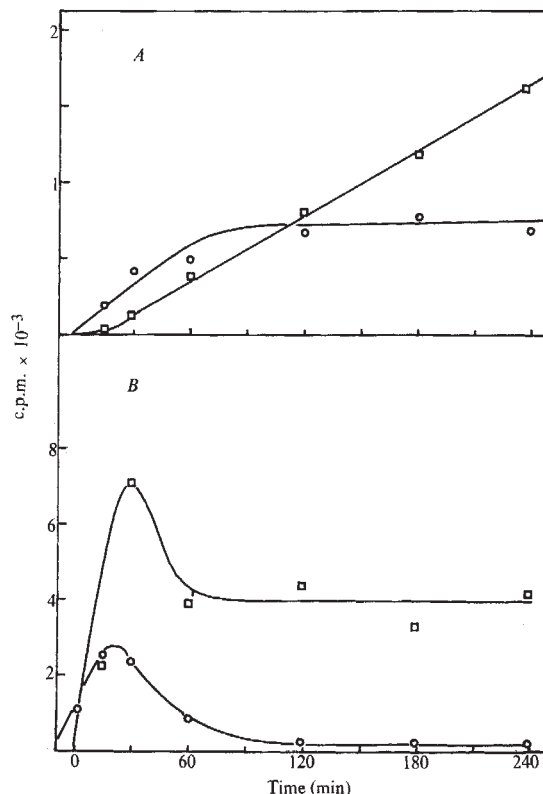


Fig. 2 A, Incorporation of ¹⁴C (○) and ³H (□) into protein bound aspartate plus asparagine; B, incorporation of ¹⁴C (○) and ³H (□) into soluble aspartate.

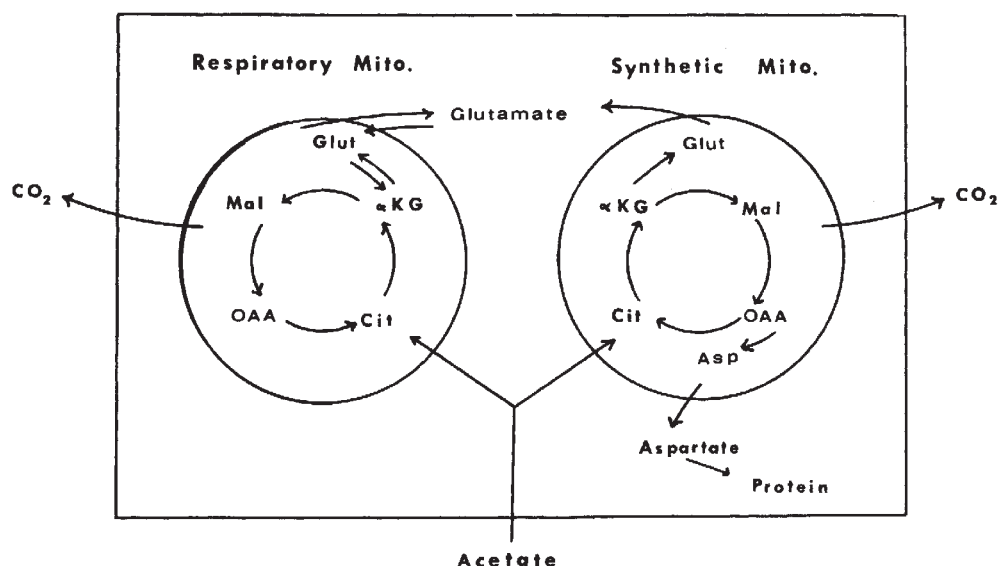


Fig. 3 Model proposing two populations of mitochondria in higher plant cells.

ideas have been advanced regarding the mitochondria in animal cells⁴. In the work reported here, double labelling techniques were used to elucidate further the presence and possible role of a heterogeneous population of mitochondria in plant cells.

Suspension cultures of Paul's Scarlet rose were grown as previously described¹. I used cells and medium removed from 8-day-old batch cultures undergoing logarithmic growth. To ensure sterility each suspension was plated separately on Difco Bacto nutrient agar and incubated at 25° C for 96 h.

A 10 min pulse of 20 μCi of ^{14}C -U-acetate (51 $\mu\text{Ci}/\mu\text{mol}$) was provided to 10 g of cells (Fig. 1), followed by a 240 min chase, during which the cells were exposed to 40 μCi of ^3H -U-acetate (1 $\mu\text{Ci}/\mu\text{mol}$). During the pulse period the cells were aerated in 20 ml. of growth medium (pH 5.5) held in a 60 ml. coarse fritted glass filter funnel connected to an air line. After the pulse, the medium was drawn off through the bottom of the funnel, and the cells were washed several times with sterile growth medium. Approximately 1 g of cells was removed and homogenized in 15 ml. of 80% (v/v) ethanol to serve as the zero time sample. Another gram of tissue was transferred to a 15 ml. funnel connected to a CO_2 trap, so that the progress of $^{14}\text{CO}_2$ could be followed. The chase period was then initiated by aerating the cells in both funnels in sterile medium (pH 5.5) containing ^3H -U-acetate. At appropriate intervals, 5 ml. samples were pipetted from the 40 ml. of suspension held in the larger funnel.

Cell constituents were extracted from each sample, and the distribution of ^{14}C and ^3H was determined as previously described¹. Corrections were made for quenching. Since the pipetting of suspensions did not yield samples of uniform weight, the protein amino-acid content of each sample was used to express the observed data in terms of equal sample weights (1 g).

During the pulse period, ^{14}C -acetate was taken up rapidly (Fig. 1), and entered the TCA cycle as indicated by incorporation of ^{14}C into citrate, succinate, malate, and by evolved CO_2 . The level of ^{14}C in malate and the rate of $^{14}\text{CO}_2$ evolution remained constant during the later stages of the chase period. Therefore, ^{14}C continued to pass through the TCA cycle even though the exogenous supply of ^{14}C -acetate had been removed. The source of this radioactivity was ^{14}C which had been temporarily sequestered in other metabolites during the pulse period, primarily in ^{14}C -glutamate¹.

Carbon-14 accumulated in soluble aspartate during the pulse period and the first 15 min of the chase (Fig. 2); thereafter it declined steadily. Some of the soluble ^{14}C -aspartate entered protein. After 30 min of the chase period, there was no net synthesis of ^{14}C -aspartate (Fig. 2), although ^{14}C -labelled

metabolites continued to be oxidized in the TCA cycle during the subsequent 210 min (Fig. 1). Thus, exogenously supplied ^{14}C -acetate which entered the cells during the pulse period was metabolized in the TCA cycle, and a portion of the oxaloacetate generated was converted to ^{14}C -aspartate. Carbon-14, however, temporarily sequestered in other metabolites during the pulse period, primarily ^{14}C -glutamate, did not give rise to ^{14}C -aspartate on re-entry into the TCA cycle during the chase period.

^3H -Acetate provided during the chase period was taken up gradually (Fig. 1). After 15 min tritium was present in all TCA cycle intermediates examined. It accumulated in malate during the chase period (Fig. 1), demonstrating the continued entrance of tritium into the TCA cycle. ^3H -Aspartate accumulated initially and then remained at a relatively constant value during the remainder of the experiment (Fig. 2). Throughout the chase period there was a steady incorporation of ^3H -aspartate into protein. After 60 min there was a 35% increase in the total ^3H -aspartate (soluble plus bound) present in the cells. During this period of net gain in ^3H -aspartate there was a 41% decrease in ^{14}C -aspartate although ^{14}C continued to pass through the TCA cycle at a steady rate as indicated by the constant rate of $^{14}\text{CO}_2$ evolution (Fig. 1).

The results reported here have been interpreted in the form of a working model shown in Fig. 3. Two populations of mitochondria are proposed; one associated primarily with respiration (respiratory mitochondria), and the other with the provision of carbon skeletons for subsequent biosynthesis (synthetic mitochondria). Both types of mitochondria are permeable to acetate, and possess a complete tricarboxylic acid cycle. On the basis of the present data, it is not clear where glutamate is synthesized; therefore, the model shows it as being synthesized in both types of mitochondria. A distinguishing feature of the respiratory mitochondria is that endogenously synthesized glutamate only enters the TCA cycle present in that compartment. A second distinction between the two types of mitochondria is that oxaloacetate is only converted to aspartate in the synthetic mitochondria.

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Energy Substrates and Culture of Single Cell Rabbit Ova to Blastocysts

THE metabolic requirements of the preimplantation stages of mammalian development are difficult to study *in vivo*. The use of synthetic culture media capable of maintaining growth of the fertilized ovum is a useful technique for this problem. Continuous culture from the single cell fertilized ovum to the blastocyst stage in synthetic media has already been achieved for the mouse¹ and rabbit².

In the only previous report of culture of single cell rabbit embryos to blastocysts in a synthetic medium, the medium contained glucose in all cases, and addition of pyruvate improved blastocyst development². Growth of the embryos in a medium without carbohydrate substrates was not studied. We report here investigations to determine whether the single cell rabbit ovum required glucose or pyruvate for development. A very high percentage of single cell ova developed to blastocysts in the absence of glucose and pyruvate and the addition of these substrates did not significantly promote growth to the blastocyst stage. This indicates that either endogenous sources or non-carbohydrate compounds in the medium can supply the energy for growth.

Table 1 Effect of Pyruvate and Glucose on Development of Hatched Blastocysts from Single Cell Rabbit Ova in Culture

Carbohydrate added	No. of single-cell ova tested	Hatched blastocysts %	Diameter (μm)*
None	42	81.0	383±25
5×10 ⁻⁴ M pyruvate	42	71.4	483±27
1×10 ⁻² M glucose	42	64.3	376±30
Glucose+pyruvate	42	81.0	373±24

*Figures are mean blastocyst diameters±s.e.

There was a significant interaction ($P<0.025$) of glucose and pyruvate on hatched blastocyst diameter. No other orthogonal comparisons were significant.

The experiment was a 2×2 factorial arrangement, in which 5×10⁻⁴ M pyruvate and/or 1×10⁻² M glucose were added to a basal medium (Table 1). The glucose concentration was the same as that previously reported² and the level of pyruvate was similar to that present in the Fallopian tube of the rabbit (2×10⁻⁴ M) (see ref. 3). The basal medium contained salts, amino-acids, vitamins, trace elements, antibiotics and 1.5% crystallized bovine serum albumin (BSA) as described previously⁴. All media were maintained at 270 mOsm by adjusting the NaCl concentration⁵.

Superovulation and insemination of the donor doe and ovum collection and handling were carried out as outlined by Kane and Foote⁴ but with human chorionic gonadotrophin (HGG) used as the ovulating hormone, and ova were collected 20 to 21 h later. Abnormal ova were discarded and the remaining ova were washed by passing them through 3 ml. of substrate-free medium four times, randomly divided equally between the four treatments and placed in culture in droplets of 0.5 ml. of medium under 10 ml. of paraffin oil ('Merck-ampoule' grade) in tissue culture dishes.

One drop was used per treatment for ova collected on one day. Culture dishes were maintained in a gas phase of 5% CO₂ in air at 37–38° C. Ova were examined daily for five days at magnifications of ×40 and ×100, and the number of blastocysts in each drop which "hatched", or escaped from the zona pellucida, was recorded on days 4 and 5 of culture. The diameters of the hatched blastocysts were measured on day 5.

Data on numbers of hatched blastocysts were analysed by orthogonally partitioned chi-squares and those on blastocyst diameters by variance of analysis⁶. Numbers of blastocysts per treatment were equalized by randomly eliminating blastocysts from some treatments for statistical analysis of blastocyst diameter.

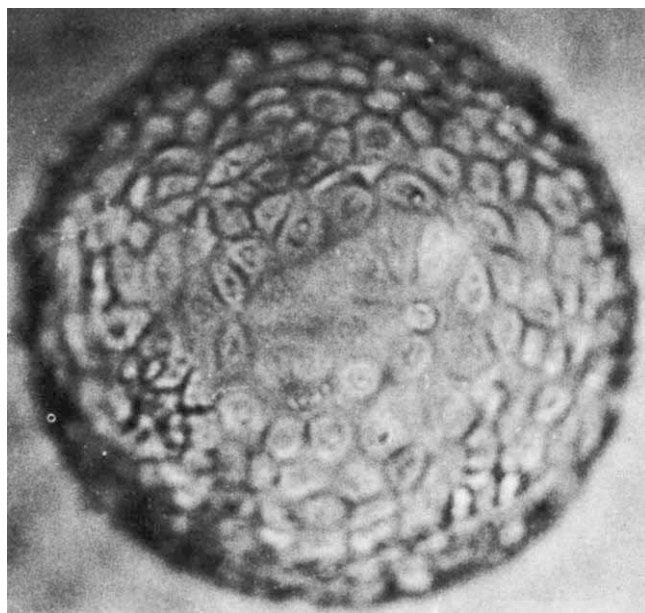


Fig. 1 Hatched blastocyst developed from single-cell rabbit ovum after about 110 h culture in the basal medium without carbohydrate energy sources. Diameter of the blastocyst is about 420 μm. Cells with nuclei are visible and part of the inner cell mass may be seen in the lower left-hand corner. Some parts of the blastocyst are out of focus because of its size and spherical shape. (× c. 195.)

In all treatments a very high proportion of blastocysts hatched (Table 1); the overall mean of 74.4% of 168 single cell ova becoming expanding blastocysts and escaping from their zonae in culture is a marked improvement on the previous results of Kane and Foote² with only 29% of expanding blastocysts and about 1% hatched from the zonae. This improvement may be due to the breed of rabbit or to technical differences. New Zealand rabbits were used for these experiments, while Dutch Belted rabbits were used by Kane and Foote². In our previous work² trays with 'Dow Corning Medical Fluid 360' were used instead of the plastic tissue culture dishes with paraffin oil. The grade of paraffin oil used appeared to be particularly suitable for ovum culture because there was no problem of droplets of medium flattening and running together under the oil. Such flattening of droplets has previously been associated with poor growth of embryos³.

Because rabbit blastocysts, unlike some rodent blastocysts, have not been observed to escape from the zona *in vivo*⁷ we intend to establish the necessity for the zona in blastocyst viability by transferring expanding blastocysts before and after hatching to synchronized recipients.

The most significant fact to emerge from the experiment is that a very high proportion (81%) of single cell ova developed to blastocysts in the absence of energy substrates such as glucose or pyruvate (Table 1). The development of ova to blastocysts was not significantly affected by the addition of glucose or pyruvate. Rabbit ova may be able to utilize the amino-acids or the BSA present in the medium as energy sources; alternatively they can rely on endogenous sources of energy, by contrast to the mouse where an absolute need for either pyruvate or oxaloacetate to support cleavage of the single cell stage has been reported¹. Our results agree with those of Brinster⁸ who demonstrated that an amino nitrogen source such as BSA, oxidized glutathione or certain single amino-acids when added to a simple salt type medium supported cleavage of 2-cell rabbit ova to morulae and that carbohydrates were not necessary.

There was no significant main effect of pyruvate or glucose on the expansion of the hatched blastocysts but there was significant interaction of pyruvate and glucose (Table 1). This was due to the fact that addition of 5×10⁻⁸ M pyruvate alone

increased mean blastocyst diameter from 383 μm to 483 μm but further addition of 10^{-2} M glucose appeared to inhibit this effect. Fig. 1 shows a hatched blastocyst developed in the basal medium.

That carbohydrates are not essential for development of single cell rabbit ova affects the study of metabolic patterns in these embryos because the existence in the early embryo of different parts of the tricarboxylic and glycolytic pathways cannot be studied. This approach has been very successful for the mouse ovum^{9,10}, but it seems that in the rabbit radioisotopic and inhibitor approaches rather than on nutritional studies must be used, at least for the pre-blastocyst stages.

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Trackways of Tetrapod Vertebrates from the Upper Devonian of Victoria, Australia

THREE distinct trackways of tetrapod vertebrates were discovered late in 1971 by one of us (N. A. W.) in strata of the Genoa River Beds at lat. 37° 19' S, long. 149° 23' E, in eastern Victoria, Australia. The Genoa River Beds were considered by Dun¹ on the basis of plant fossils and Hall² on stratigraphic relationships to be of Upper Devonian age. Hall suggested they are the non-marine lateral equivalents of the Merimbula Formation which contains a marine fauna indicative of the Upper Devonian, most probably Frasnian. Following the discovery of the trackways, J. G. Douglas (Victorian Mines Department, unpublished report 1972/3, 1972) located an assemblage of Upper Devonian plants in the Genoa River Beds at approximately the same stratigraphic horizon as the trackways and within 850 m of them. The flora includes sphenopsid stems of possibly more than one species and fragments of fernlike leaves referred to by Dun¹ as *Archaeopteris howittii*.

The only previous report of pre-Carboniferous vertebrate trackways is the speculation by Willard³ that enigmatic marks on some Upper Devonian shales from Pennsylvania were caused by tetrapods, but this has never been verified or widely accepted. Thus, the Genoa River trackways are the earliest yet recorded for limbed vertebrates. They also constitute the earliest recorded occurrence of tetrapods on the Australian landmass⁴.

In the following descriptions we use the restricted definitions of terms used to describe tetrapod trackways given by Peabody⁵. All three trackways occur on the same bedding plane in a purplish-brown very fine-grained sandstone that bears other trace fossils, some of which may represent invertebrate trails.

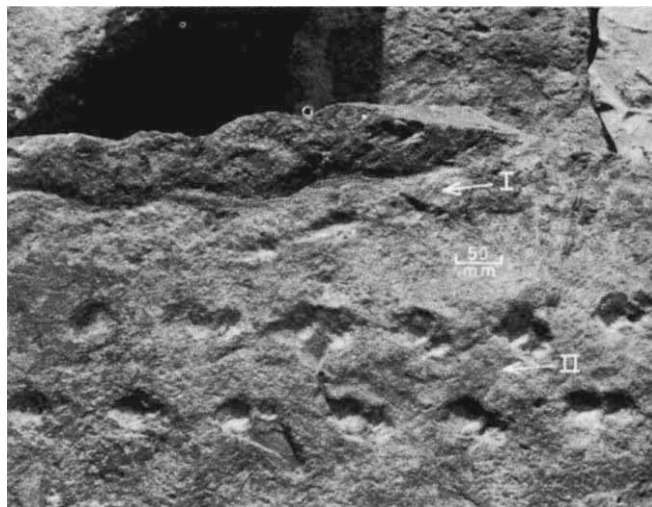


Fig. 1 Trackways I and II. Arrows indicate direction of movement.

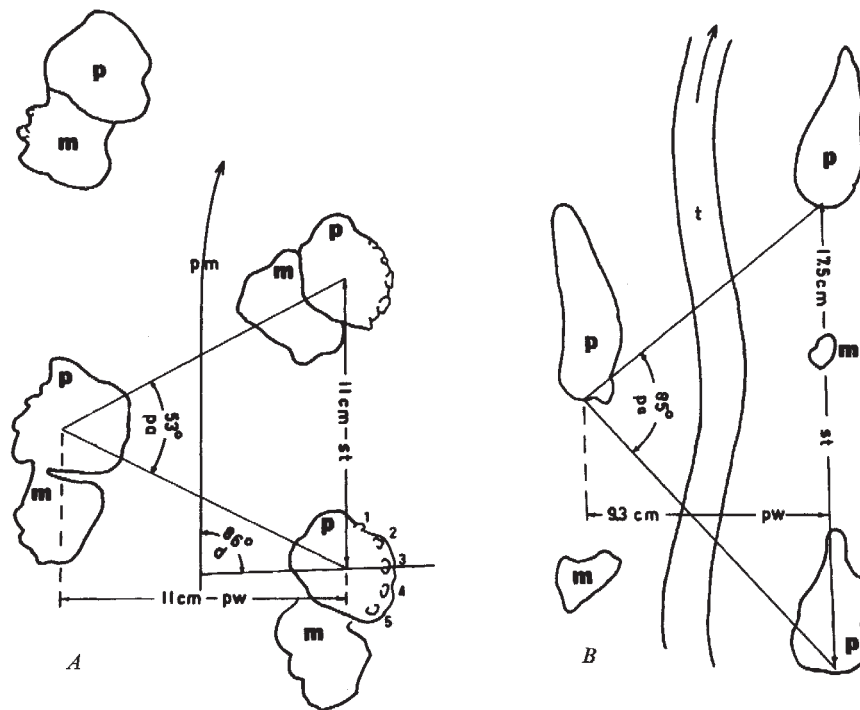
Trackway I (Fig. 1) is 1.1 m long and is a remarkably clear series representing ten left sets of impressions and nine right sets. The impressions are original imprints (positives) and are filled to varying degrees by a comparatively coarse-grained sandstone.

Measurements on the clearest three consecutive sets of impressions are shown in Fig. 2A. Of interest is the wide divarication of the pes from the midline (86°), the relative width of the trackway as expressed by the pace angulation (53°), and the absence of a tail or body mark indicating support by the limbs. In all sets of impressions in this trackway the pes has overstepped the manus and in most sets it has partially obscured the leading edge of the imprint of the manus. This suggests a condition of secondary overlap as defined by Peabody⁶, and helps to fix the degree of coupling and the distance between the pectoral and pelvic girdles. The gleno-acetabular distance would be slightly less than 22 cm, that is twice the stride, and the trunk region slightly more. If the tail is considered to be of approximately equal length to the trunk, and the head about one half this length, which are common proportions in early tetrapods, the overall length of the animal that made trackway I would be near 55 cm.

In trackway I the general outline of the pes, including digits, can be discerned in at least six of the impressions; others appear to be equally well preserved but require further preparation. The pes (Fig. 3) has five digits, none with an appreciable free length, but with interdigital tissue that has depressed the substrate leaving only indications of the apices of the digits, or possibly terminal pads. The least distorted impressions of the pes are 3.5 cm wide measured at 90° to the central axis, and are 0.5 cm deep. The manus is smaller than the pes and clearly possesses three digits, but the possibility of it having four or five cannot be discounted at this stage.

Trackway II (Fig. 1) is adjacent to trackway I on the same rock slab and passes in the same direction. It consists of four right and four left impressions of hindfeet, which were dragged forward in the substrate resulting in elongate tracks showing no detail of foot structure. There are three impressions of the right manus but only two of the left, and there are two spaces between the imprints of the left pes where there is no trace of the manus. There is an undulating tail or body mark. Four consecutive impressions of the hindfeet and three of the forefeet are shown in Fig. 2B. The pes has not stepped onto the imprint of the manus as in trackway I, but the two alternate regularly. This difference, in conjunction with the longer stride (17.5 cm), the greater pace angulation (85°), the shorter width of pace (9.3 cm), and the fact that the manus impression is sometimes missing, could indicate that the individual responsible for this trackway was partially using body and tail

Fig. 2 Portions of (A) trackway I and (B) trackway II. Measurements in I are from estimated centre of pes impression; *d*, divarication of pes from midline measured through third digit; *pa*, pace angulation; *pm*, midline of pace; *pw*, pace width; *st*, stride; *t*, tail drag; *p*, pes; *m*, manus; 1-5, tentative number ring of digits.



undulations for locomotion. This would result in a greater apparent arc in each limb movement and a reduced width, as well as account for the drag of the hindfeet.

Trackway III is 5 m from the other two on the same bedding plane and consists of the original imprints (positive) and the cast of them (negative) on a counterslab. It is 1.05 m long with six complete sets of impressions on the left side and five imprints of the pes and three of the manus on the right. No detail of foot structure is preserved and each impression shows evidence of some drag and slumping. There is no tail or body mark. The relevant measurements are stride 19 cm, pace angulation 80° , and width of pace 10.5 cm. The impression of the pes alternates with that of the manus and in no case obscures it. This type of overlap in conjunction with the larger stride and width of pace indicates an animal with a greater degree of coupling than that responsible for trackway I, and its overall length is calculated to be near 90 cm.

These are the earliest known terrestrial tetrapod trackways.

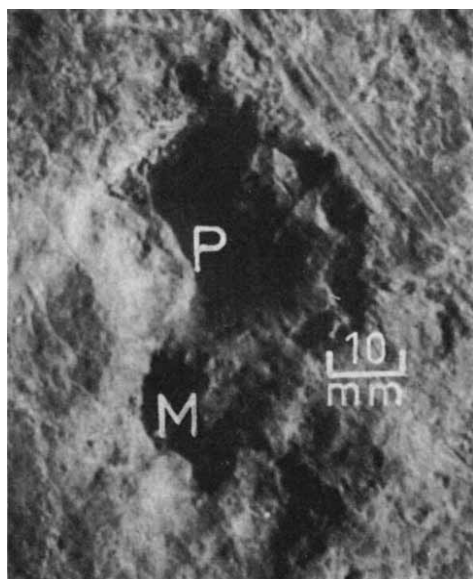


Fig. 3 Impression of the pes from trackway I. M, manus; P, pes.

They coincide in time with the earliest known Amphibia, the Ichthyostegalia, which are recorded only from the Upper Devonian Old Red Sandstone of Greenland. The best described genus of the Ichthyostegalia, *Ichthyostega*, is slightly less than 1 m in length, possesses short limbs, and has a pes with five short digits and a width of about 3.5 cm⁷. This length is near that suggested for the animals responsible for these trackways and the width of the pes of *Ichthyostega* corresponds closely to the size of these fossil impressions.

The divarication of the pes from the midline (86°) in trackway I, which is the only one where this can be determined, is greater than recorded for any later amphibian, where the hindfeet are forwardly directed with little or no divarication⁸. This trackway, with the hindfeet directed outward, indicates there was little axial rotation of the limbs and lends support to a suggestion by Morton⁹ that this should have been the case in the most primitive tetrapods, a suggestion which has lacked supporting evidence until now.

Because of the excellent preservation of detail in trackway I, it will be possible to analyse the gait and distribution of forces during the propulsive stroke, and to provide an adequate description for purposes of nomenclature.

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BOOK REVIEWS

Human Children as Animals

An Ethological Study of Children's Behaviour. By W. C. McGrew. Pp. xiv+257. (Academic: New York and London, March 1972.) \$10.50.

THIS book is an expression of an emerging trend to look at human behaviour in much the same way as ethologists of the early 'thirties began to look at animal behaviour. In particular, it is a step in the process of constructing an "ethogram" of our own species; the step characterized by pinpointing, in the seemingly bewildering variety of movements that constitute "behaviour", a core of relatively simple "elements" which can be seen with fair regularity and constancy in all individuals of a species or population. To discern such more or less constant elements is particularly difficult in one's own species where familiarity suppresses curiosity, and where variety is more conspicuous than constancy. It is even more difficult to communicate, in an unambiguous way, what the elements look like.

When one approaches human behaviour from this angle, one soon finds oneself faced with two additional tasks: how to allocate one's method its proper place in the wide spectrum of already existing disciplines of human behaviour; and what to do with one's observations; how to make scientific sense out of one's data; how to manipulate them so that they can help solve particular problems.

Dr McGrew's book falls roughly into three parts which reflect these considerations. The first chapter, called "Historical Review", attempts to define the relations between ethology and some other human sciences: developmental psychology, social and clinical psychology, and anthropology and sociology; it also has a section on aggression and one on nonhuman primate studies. The second part contains reports on some specific, original work done by the author. The third part, which is a substantial inventory or catalogue of the "elements" recorded by the author, is placed in Chapter 4, following a statement of his research problem and his method.

As could hardly be expected otherwise (because each of the disciplines

mentioned is so different from the others), the historical review does little more than highlight the confused, searching state of the various fields, and point out the need for straightforward descriptions of behaviour.

The most valuable part of the book seems to me Chapter 4, which contains descriptions and some illustrations of the well over 100 elements that students of children's behaviour have begun to single out and characterize. Although, as the author points out, there is considerable correspondence with comparable catalogues given, for instance, by Blurton Jones and Grant, the classification is still being developed. It is to be hoped that this kind of work will in the not too distant future lead to an agreed-on detailed inventory, in which the "elements" will be carefully described and above all very clearly illustrated, for no description can convey what one carefully selected picture can. For this, close collaboration with, for example, Eibl-Eibesfeldt will be essential.

In the more factual part of the book Dr McGrew reports on some specific studies which are based in essence on counting out the occurrence of a number of elements in nursery school children observed in different circumstances. Presumably these five factual reports are intended as examples of the applicability of this type of data-recording. Apart from the fact that this section contains much that has already been published elsewhere in much the same form, I find it rather disappointing. The use made of the method is very limited. Since the author has shrugged off rather contemptuously and indiscriminately practically everything that animal ethologists have tried to contribute to our understanding of human behaviour—dismissing Lorenz's writings as no more than speculative extrapolation, and Morris's books as "pop ethology"—one would have expected him to present something more substantial and more imaginative than this section contains. For example, neither the grouping of elements into more comprehensive systems of either causal or functional

affinity, nor the comparisons with non-human primates, nor the placing of the elements and their changes with time in the context of social integration really comes off the ground; what we get is little more than some rather restricted raw material. This would not matter in a series of single, factual research reports which ultimately lead up to some kind of synthesis, but to suggest, as both the book's title and its theoretical chapters do, that this type of work represents the essence of "the" ethological method is surely misleading — ethology, even where it refrains from premature explanation, interpretation and speculation, is more exciting than these rather pedestrian results of conscientious counting. In a book carrying this title one could have expected a more problem-oriented historical introduction, a clearer statement of the author's ultimate aims, and more penetrating discussions of the sense of what is described. Perhaps this is asking too much from such an early attempt; but one has a right to find something more nourishing in a book of xiv+257 pages, published by Academic Press. While the book does contain some interesting facts and thoughts, it disappoints by its lack of coherence, of discipline, of clarity, and also of humility. N. TINBERGEN

Practical Astrodynamics

Fundamentals of Astro-dynamics. By Roger R. Bate, Donald D. Mueller and Jerry E. White. Pp. xii+455. (Dover Publications: New York; Constable: London, January 1972.) £2.25.

THE end-product of evolution in a rather unusual environment, such as the marine iguana of the Galapagos, often proves to be interesting. This book records the course in astrodynamics that has evolved over the years at the United States Air Force Academy, and it is interesting to see how an academic subject has been modified by practical imperatives. The departures from the norm, which turn out to be quite exten-

sive, give the book a distinctive flavour which fully justifies its publication. A notable feature is the treatment of the "Gauss problem", determining an orbit from two (radar) positions and times, a reminder that the USAF has the uncomfortable task of identifying the Earth impact points of all objects sighted by radar. The solution of Kepler's equation is also fully discussed, with emphasis on the use of "universal variables" which are valid for both the ellipse and the hyperbola: this bias is logical, too, for newly-sighted objects do not carry placards advertising their eccentricities. Other chapters cover ballistic missile trajectories and interception; lunar trajectories, with emphasis on the useful patched-conic approximation; and interplanetary transfer orbits, including the problems of plane-changing. There is also a section on space surveillance, giving the most detailed account yet published of the USAF tracking network.

The layout of the book has obviously been considered with care, and the general appearance is pleasing: the diagrams are well drawn; many of the symbols are clean and elegant in design; and it is useful to have rectangles enclosing the most important equations, though traditionalists may wince. Unfortunately, however, the detailed printing of the mathematics is not far short of a typographical disaster. Hundreds of corrections are needed where symbols run together, suffixes are unlabeled, diagrams and text are inconsistent, alignments are incorrect or spacing excessive. Worst of all are the minus signs: a good healthy minus, about 2 mm long, graces the early pages of the book; but on page 115 we meet a mini-minus, about 600 μ m long, and difficult to distinguish from a dot. After a running battle lasting about sixty pages, the mini-minus finally ousts the mega-minus, to the dismay of all but short-sighted readers. The units are as mixed as the minus signs, and readers need to be alert to recognize that km signifies kilometres but nm stands for nautical miles. In spite of these typographical troubles, the text is very readable, being written in a pleasant style, with some interesting historical digressions.

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DNA and All That

The New Biology. By Graham Chedd. Pp. xiv+306. (Basic Books: New York, 1972.) \$7.50.

"The New Biology" has come to describe the approach to living systems at the molecular level which has proved so successful in the past decade, not just in molecular genetics but also in cell biology, immunology and neurobiology.

These are the topics discussed in Graham Chedd's new book, which takes a narrative approach to this subject. The style and level of discussion may fairly be termed "popular" although the content of the book seems to be intended for students somewhere between school and university.

The first part of the book is concerned with the development of modern genetics, the discovery of the genetic role and structure of DNA, and the synthesis of nucleic acids and proteins. This is well written, with its emphasis on the personalities involved and their key experiments, and provides a straightforward account of the development of molecular biology. The book is written largely *post hoc ergo propter hoc* and an air of ineluctable discovery accompanies much of the discussion.

The next section of the book discusses the structure and evolution of proteins and again provides a history of the successful experiments performed on the structure of macromolecules. Any errors in the first part of the book tend to be minor and of emphasis rather than in fact, although the unwatchful reader may on occasion be misled by the author's habit of presenting conclusions two or three pages before the important qualifications which should be attached to them.

The discussion of more recent research into control mechanisms in bacteria and in higher organisms is on less sure ground. Too much emphasis is placed on translational control and there are some misleading comments on allosteric regulation of enzyme activity and on transcriptional controls in bacteria. Chedd's enthusiasm for sigma factors as control proteins, however, and his acceptance of Bonner's experiments with chromosomal RNA may perhaps seem uncritical only as the result of events which have taken place since the book was written.

The account of cellular research in higher organisms is more compressed than the discussion of molecular biology; and the sense of perspective which is to be found in the early part of the book is missing later. The final chapters are concerned with cancer—and in particular with tumour viruses—ageing and the brain. Much of the discussion in the later parts of the book is too brief to do justice to its subject matter, especially the last section on the brain.

Narrating the future is no easy task and the personal narrative approach is less successful when used to project future trends than when applied to history. That "Watson is personally convinced that the work presently being done on polyoma and SV40 will produce a molecular understanding of cell transformation within the next five or

ten years" may be interesting; but it would be more useful if the author were to tell us what conclusions he himself draws from his previous discussion and why.

The main emphasis of the book lies, in spite of its title, in classical molecular biology. Indeed, for a readable account of the crucial experiments in molecular biology, this is a useful book for the beginner and Chedd has fulfilled his principal objective, telling a simple story concisely and accurately. Discussion is at its weakest on very recent research and future trends; the attempt to make the book very topical has perhaps proved too difficult. The first part of the book, again, may be interesting to the layman, but the subsequent chapters are likely to prove too complex. Although the index is adequate, the surprising lack of any bibliography must restrict its use for the student.

BENJAMIN LEWIN

Ice Age Geology

Glacial and Quaternary Geology. By Richard Foster Flint. Pp. xii+892. (Wiley: New York and London, June 1971.) £10.95.

Glacial and Quaternary Geology by Richard Foster Flint sums up his view of the subject on his retirement after a lifetime spent studying this field. As a work of synthesis, the volume represents a remarkable achievement both in the breadth of its coverage and for the selectivity with which the author has, over the years, sifted and evaluated contributions from the various specialized fields which relate to his general study of the Quaternary.

Since the publication in 1957 of his earlier general work *Glacial and Pleistocene Geology*, substantial advances have been made in a number of fields due to the developing use of techniques such as ^{14}C and K/Ar dating, coring of the ocean bed and of ice sheets, and the use of oxygen isotopes to study climatic history. Flint claims that the present volume contains about 80 per cent new material, and a comparison with his earlier book bears this out. The bibliography, for example, has increased from 34 to 63 pages and spot checks of 200 references show that 63 per cent are to papers which appeared since his 1957 volume was written. A check of 100 references in that volume showed that about half had been dropped in the new volume, including half the references to his own earlier work. As a guide through the literature of a vast and complex subject, the present volume, at once comprehensive and discriminating, is invaluable.

The book is, however, more than a review of its subject. It is also a text-

book providing an introductory discussion of each topic and its relationship with the other factors involved. The first half of the book is concerned mainly with processes. After a broad history of ideas in the two opening chapters, aspects of physical glaciology and glacial geology occupy the next six before the author moves on to topics such as aeolian features, fluctuation of sea level, glacial-isostatic deformation and pluvial features. Basic methods of stratigraphy and geochronology are discussed in principle before the presentation of detailed results of such studies on a regional basis. These studies, which occupy some 250 pages, cover North America in most detail, followed by Europe and then other parts of the world. A relatively short chapter on stratigraphy of deep sea floors is of importance, then follow two chapters on Quaternary fossils, bringing in the biogeographic approach in relation to land bridges.

As a broad survey the book is excellent, although on the treatment of individual topics any reviewer is likely to suggest that too little attention is given to his own field of interest. Chapter 3, for example, deals summarily with glacier mechanics, devoting only two or three paragraphs to each major topic of interest to glaciologists today. Although this is typical of most books on glacial geology, it seems a pity, as a more thorough understanding of physical glaciology could be of considerable benefit to students of glacial geology. For example, in the discussion of fluctuations of sea level, too much caution is shown towards use of glaciological knowledge when considering problems such as the volume of ice sheets at the maximum glaciation, or the behaviour of ice sheets during interglacial periods. In other respects the chapter on fluctuation of sea level provides an excellent introduction to a complex and confusing subject; and the treatment of problems connected with movement of the Earth's crust beneath ocean basins draws attention to an important factor which is usually neglected.

In the final chapter—"The Problem of Causes"—the author considers the broadest aspects of the subject. The general problem is first set out in terms of the relation of climate to solar heat. Reliable data on climate over the past few hundred years are considered in relation to sunspot cycles and in relation to ideas such as those of Milankovich. Theories are then grouped into six main types of process and each main idea is given some discussion. A few individual hypotheses are discussed briefly, such as those based on variations of circulation and ice cover of the Arctic Ocean (Donn and Ewing) and surging of the Antarctic ice sheet (Wilson), and pertinent reasons given for

not adopting them. This leaves the solar-topographic concept as outlined in Flint's earlier books as the effect likely to feature most prominently in the final explanation of ice ages. It must be a source of satisfaction to the author that the accumulating evidence still points to the basic importance of some type of solar-topographic hypothesis, since it has been emphasized consistently in his three successive major volumes on the subject in 1947, 1957, and 1971.

The book is well produced with relatively few mistakes; every student of the Quaternary will wish to possess it.

G. DE Q. ROBIN

The Proper Study

Comparative Genetics in Monkeys, Apes and Man. Edited by A. B. Chiarelli. (Proceedings of a symposium on Comparative Genetics in Primates and Human Heredity, held at Ernice, Sicily, July 1970.) Pp. x+346. (Academic: New York and London, December 1971.) £5.50; \$16.50.

THERE is a natural tendency for grant-giving bodies to be particularly generous in supporting research that has some obvious relation to human problems. Unfortunately the obvious relations are not always the most interesting or productive. When there is more money, there may be less competition for it, a state of affairs that could serve to explain the curious correlation known as the Rothschild Inversion. It can briefly be summarized as follows: the nearer one approaches the subject of man himself, the higher is the proportion of scientific rubbish.

This book, alas, is a good example. Certainly we need to know more about our immediate relatives in the animal world; certainly the comparative method can be a powerful one; but there are pitfalls, and the authors of these essays fall into a lot of pits. In a book offered for general sale we may reasonably expect that reports of comparative studies should say more than: "Look, the orang-utan has got one too!" They should attempt some general conclusions. Only about half the essays make the attempt, and those that do so tend to go to the other extreme.

A good example is the paper by Weiss and Goodman, in which fifteen pages of speculative argument are based on a survey of protein variation in three samples of Asian macaques. Most of the argument concerns a difference in average heterozygosity between *Macaca* and *Drosophila* that is almost certainly statistically insignificant. Goodman and his colleagues also contribute a paper on the evolution of primate genes and

proteins. They claim priority over Kimura, King and Jukes for the theory that divergent protein evolution is largely due to the accumulation of neutral mutations. They also propose that evolution has slowed down in the primate line. The proposition is certainly untrue of intellectual and morphological characters, and their theory seems to be incompatible with what is known about the extent of enzyme polymorphism in man.

There are essays on dermatoglyphic traits, the tasting of phenylthiocarbamide, transplantation antigens, blood groups, and immunoglobulins. The final paper, by Chiarelli on comparative cytogenetics, contains a statement that deserves quotation without comment: "... meiosis represents a barrier which prevents exchanges between diverging genetic systems. Its function... is therefore to stabilize the genetic information defining a given species...".

Two essays, one by the Berrys on epigenetic polymorphism, the other by Sullivan on haemoglobins, stand above the rest. They have interesting things to say, and say them with clarity and reason. Two good papers, however, are not enough.

The book can be recommended to rich librarians and Lord Rothschild, but to the latter only as a warning.

BRYAN CLARKE

Electromagnetism

l'Energie Electromagnetique Materielle et Gravitationnelle. By R. L. Vallee. Pp. vi+138. (Mason et Cie: Paris, 1971.) 50 francs.

MANY scientists will read this book with pleasure and even with excitement; others may be sceptical, but all should read it with interest and benefit. The author deplores the abstract nature of modern physical theories such as relativity and quantum mechanics, and suggests that if we are to control our environment we must understand it, and if we are to understand it we must have a more concrete physical model of phenomena even on a microscopic scale. He regards it as a grave fault of existing theories that they accept the mathematical fiction of empty space whereas space in the real world is filled with fields of various kinds. He therefore postulates the existence of an energy propagation medium and assumes further that the energy it contains can be expressed as electromagnetic energy. A stationary frame of reference can be defined in terms of the state of the momentum in the medium. It is then argued that if the electric field is never to become infinitely great there must be

a limiting value calculated to be about $38.67 \times 10^{15} \text{ V m}^{-1}$, at which the properties of space are changed. There are regions of finite dimensions in which the divergence of the electric field is not zero but has the integrated values $+q$ and $-q$ ($q = 1.6 \times 10^{-19}$ coulombs). It is in such divergent zones that photons are produced. From these assumptions and with the aid of Maxwell's equations, and the Lorentz transformations used simply as a mathematical operator, but without using the relativity principles, the author derives the fundamental equations of quantum mechanics, wave mechanics and gravitational fields. He thus obtains the same results as existing theories but gives a clearer physical picture of the phenomena, including models of the photon and electron. The theory also leads to some new results. The velocity of light and Planck's constant are not universal constants but limiting values of more fundamental quantities which depend on the material matter in the medium. For example, the velocity of light has the limiting value c in intersidereal space but a value 0.1 m s^{-1} less than this at the surface of the Earth.

A transverse electromagnetic field is accompanied by a gravitational field and there is a simple relationship between gravitational potential and the velocity of light.

The book is clearly written and is mainly descriptive in character, the author's aim being to make it readable and comprehensible to both experimental and theoretical scientists. It would have been improved by the addition of an index and a list of symbols.

L. ESSEN

Amines in Neurones

6-Hydroxydopamine and Catecholamine Neurones. Edited by T. Malmfors and H. Thoenen. Pp. 368. (North Holland: Amsterdam and London; Elsevier: New York, July 1971.) \$22.50.

COMPOUNDS with novel properties crop up all too often in the pharmacological literature, only to disappear from view with great rapidity. A welcome exception is that interesting isomer of noradrenaline, 6-hydroxydopamine. The compound has now been the subject of an exclusive symposium drawing together much of the knowledge accumulated over the period of about eight years since it was first studied, and extending it with hitherto unpublished information.

Interest was aroused when it was discovered that 6-hydroxydopamine could induce a depletion of the noradrenaline content of the mouse heart which lasted up to 30 days. This depletion was not due, at least after the first hour, to

replacement of the neuronal noradrenaline by 6-hydroxydopamine and no direct relation between amine depletion and loss of adrenergic function could be seen. Rather later the dramatic demonstration by electron microscopy that 6-hydroxydopamine caused severe morphological damage to the peripheral adrenergic nerves indicated that the drug had a unique type of action. Following injection, it could cause a long lasting sympathectomy and could really be described as a "pharmacological tool".

This intriguing possibility stimulated research into both the mode of action of 6-hydroxydopamine itself and into the action of other drugs in animals sympathectomized by pretreatment with 6-hydroxydopamine. As a result the printed output of these investigations snowballed and culminated in this present symposium with many of the major investigators brought together under one roof.

The result is a beautifully produced volume, excellently presented but, of course, at a high cost. It succeeds reasonably well in its aims, stated in the foreword, to publish new data and not just to provide a second airing for results already published. Discussion has been carefully edited down to practical dimensions by the chairmen of the various sessions and in consequence contributes rather more to the book than is often the case. There are fairly frequent typographical and other errors scattered around, but not enough to detract significantly from the overall quality. But sometimes, as a result, it is really hard to puzzle out the author's meaning. This is part of the price one must pay for a rapid publication and the international character of the gathering.

The remarkable biochemical and morphological changes produced by this drug are explored in some depth and with some duplication by the contributors. The local injection of 6-hydroxydopamine into the brain to interfere with the catecholamine containing neurones is well described. The compound, once concentrated within the neurones by their normal catecholamine uptake systems, leads to destruction of their transmitter store and functional integrity. These changes can be shown by electron microscopy and by the formaldehyde induced fluorescence technique. Even the nerve fibres themselves, which do not readily fluoresce with formaldehyde, can be revealed by the accumulation of transmitter immediately above the 6-hydroxydopamine induced lesion.

Functional studies following the production of these central lesions are not ignored, nor also are the effects of the drug on the peripheral adrenergic nervous system. It does come as a surprise, however, to find an occasional paper that

does not use the rat, and, in particular, that useful animal the chick makes an appearance.

This book is a commendable effort on the part of both contributors and editors. It is an excellent source of information and should maintain its usefulness for another year or so.

B. A. CALLINGHAM

Bilingual Bones

Atlas of Animal Bones for Prehistorians, Archeologists and Quaternary Geologists/Knochenatlas für Prähistoriker, Archäologen und Quatärgeologen. Drawings by Otto Garraux. Pp. vii+159. (Elsevier: Amsterdam, London and New York, 1972.) Dfl.90; \$28.25.

THIS bilingual (English and German) atlas, by a well-known practitioner, has a strictly limited aim—to provide, for archaeological students and excavators, illustrations of the most representative animal and human bones commonly found on excavations. One might criticize the English version in point of occasional linguistic oddity, but it is always perfectly intelligible.

Seven short introductory sections and a bibliography explain why one should preserve bones from archaeological sites, how they should be treated, how to build up a collection of comparative material and the kinds of cultural and historical results that may be obtained by their study. There is a classification of living mammals and a list of technical terms.

The illustrations do not pretend to be complete, but are confined to the principal bones, teeth, horns and antlers of horse, ox, red deer, sheep, pig, wolf, bear, beaver, hare and man—the chief domestic species and those usually hunted for food or fur, as well as being representative members of their orders. In this way, they should enable some opinion to be formed, even by non-osteologists, of the likely nature of remains belonging to species not illustrated. The wolf, for example, stands for the digitigrade carnivores, so that even members of other families should be approximately identifiable by their general resemblances.

The plates point out, in brief notes appended, some characteristic differences between the corresponding parts of the species referred to, so that even fairly fragmentary material ought to be readily recognizable.

Within its chosen limits, this book fills a decided gap in the literature available to workers in the field, and may confidently be recommended to them. The drawings are splendidly simple and clear, the text brief and much to the point and the production of high quality. Author, illustrator and publisher are all to be congratulated.

I. W. CORNWALL

CORRESPONDENCE

Taxonomy in Distress

SIR,—Your obituary notice of A. W. Stelfox (*Nature*, **238**, 175; 1972) highlights a problem which he felt acutely: the neglect of insect taxonomy. For British workers the departure of his unique collection to Washington is a loss paralleled only by that of the Curtis Collection in the nineteenth century. Stelfox was for some 25 years my valued teacher and friend, and I often worked on portions of the collection. I knew that the time spent on amassing it had prevented his working it out as he wished and that no institution in this country could guarantee to do so. As an insect taxonomist myself, I understood his keen disappointment.

I, too, am seriously concerned about the outlook for insect taxonomy in Britain. In earlier days much was done by gifted amateurs having private means. Nowadays the burden rests mainly on the professional. The British Museum (Natural History), the Commonwealth Institute of Entomology, and the universities, have all made taxonomic revisions of certain groups. However, the staff of the two former are so occupied with the demands of a world collection and routine identifications, that little time remains for research on the fundamental British and other European material. Taxonomists are rare in our universities, where the subject is generally neglected, and are burdened with ever-increasing teaching duties. Some amateurs continue to produce valuable revisions. All this, however, is a drop in the ocean. For example, the taxonomy of several huge groups of parasitic Hymenoptera, so important economically, is deplorably out of date, in some cases by a century or more. Notable progress made just

after the Second World War is clearly not being maintained. Several specialists are due to retire and there are too few recruits. This, it must be emphasized, is not just an academic problem. Ecological studies, vital to our study of the environment, are futile unless based on sound taxonomy.

Only a large scale, carefully organized scheme to supplement existing efforts, perhaps supported by our research councils, will save the taxonomic situation. Taxonomy has too long been the Cinderella of science. We owe a duty to our illustrious pioneers to see that this does not continue.

Yours faithfully,

M. W. R. DE V. GRAHAM

*Hope Department of Entomology,
University Museum, Oxford*

Pesticides and Peregrines

SIR,—There is more than propaganda to justify the belief that the persistent pesticides led to striking declines in the peregrine falcon in many countries (*Nature*, **238**, 116; 1972). The evidence has been marshalled in detail by Hickey¹ and more recently, with special reference to their sub-lethal effects on fertility, by Ratcliffe². Mr Gunn quoted the report to the Wilson Committee³; it is a pity that he did not also mention their finding in an earlier paragraph, when discussing the very marked decline in the peregrine falcon and sparrowhawk and the decreases in the populations of the barn owl and kestrel, that "the available evidence suggests that dieldrin was responsible".

Many factors operate on the populations of birds of prey, including the activities of gamekeepers and others. There is little doubt that gamekeeping helped to cause the drastic decline of

many species in the nineteenth and early twentieth centuries, or that a lessening of these activities after that led to some improvement until the early 1950s. The declines mentioned by the Wilson Committee, and attributed by them to dieldrin, occurred from 1955 onwards. In the case of the peregrine falcon, the striking fact is that populations were generally maintained despite the attentions of gamekeepers, egg collectors and falconers even in such hard pressed areas as the coast of Sussex, until the arrival of persistent pesticides. Then both numbers and breeding success declined. Since the withdrawal of dieldrin sheep dips, breeding success has improved, as the Wilson Committee noted. So has the peregrine population, although there has been little recovery yet in Wales and southern England, despite an apparent surplus of young being produced elsewhere in Britain. As Ratcliffe⁴ added, the reasons could be complex. The increase in gamekeeping in the 1960s, accompanied in some cases by the now illegal slaughter of predatory birds, may be one of the factors affecting the recovery of the peregrine, though it does not explain the observed declines in fertility. The impact, however, is probably more severe, especially in southern England, on other species of birds of prey.

Yours faithfully,

STANLEY CRAMP

*The Birds of the Western Palearctic,
32 Queen Court,
London WC1N 3BB*

¹Hickey, J. J., *Peregrine Falcon Populations* (University of Wisconsin Press, 1969).

²Ratcliffe, D. A., *J. Appl. Ecol.*, **7**, 67–115 (1970).

³Advisory Committee on Pesticides and other Toxic Chemicals, 148 (HMSO, 1969).

⁴Ratcliffe, D. A., *BTO News*, **49**, 1 (1972).

Announcements

University News

Professor Daniel Branton, University of California, Berkeley, has been appointed professor of biology at Harvard University.

The personal title of professor of geology has been conferred on Dr John R. L. Allen, University of Reading.

Appointments

Professor Colin Robinson, University of Surrey, has been appointed a member of the Electricity Supply Research Council.

Dr Eric Eastwood, director of research of the General Electric Company, and chief scientist of the Marconi Company Limited, has been appointed president of the Institution of Electrical Engineers.

Miscellaneous

The Priestley Medal of the American Chemical Society has been awarded to Professor Harold Urey, University of California.

The Meldola Medal of the Royal Institute of Chemistry has been awarded jointly to Professor G. M. Bancroft, University of Western Ontario, and Dr J. F. Kennedy, University of Birmingham.

Dr Zeilig Rabinowitz and Dr Richard Hornreich have been awarded the Sarah Zinder Leedy award for outstanding work carried out at the Weizmann Institute of Science.

The Lunar Science Institute, Houston, Texas, is attempting to build a data information bank, relating to lunar studies since 1950. Contributions, in the form of reprints, reports, photographs, maps and so on, are requested by the Institute. Material, and inquiries, should be addressed to Moon Literature Project, The Lunar Science Institute Library, 3303 NASA Road 1, Houston, Texas 77058, USA.

International Meetings

September 5, **Liquid Chromatography**, Nottingham (Society for Analytical Chemistry, 9-10 Savile Row, London W1X 1AF).

September 5-7, **Earthquake Engineering**, London (The Conference Office, Institution of Civil Engineers, Great George Street, London SW1P 3AA).

September 14-15, **Deep Fat Frying**, Norwich (Dr P. A. T. Swoboda, ARC Food Research Institute, Colney Lane, Norwich NOR 7OF).

September 18-22, **Membrane-mediated Information**, Oxford (Dr P. W. Kent, Department of Biochemistry, South Parks Road, Oxford).

September 21-22, **The Mechanical Engineer's Contribution to Process Engineering in the Food Industry**, Cambridge (The Conference Department, The Institution of Mechanical Engineers, 1 Birdcage Walk, London SW1H 9JJ).

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Department of Trade and Industry. Safety in Mines Research 1971: Fifteenth Annual Report of the Safety in Mines Research Establishment. Pp. 92. (London: HMSO, 1972.) 68p net. [107]

Comments on Plasma Physics and Controlled Fusion, Vol. 1, No. 1, January-February 1972. (Comments on Modern Physics: Part E.) Pp. 1-32. Annual subscription: Great Britain £4.18, USA and elsewhere \$11 or £4.58. Libraries, research institutions and others: Great Britain £12.85, USA and elsewhere \$35 or £14.60. (London: Gordon and Breach, Science Publishers, 1972.) [107]

Scottish Plant Breeding Station. Fifty-first Annual Report, 1971-72. Pp. 62. (Pentlandsfield, Roslin, Midlothian: Scottish Plant Breeding Station, 1972.) 25p. [107]

Proceedings of the Royal Irish Academy. Vol. 72, Section A, No. 7: Spectral Mapping Theorems. By R. E. Harte. Pp. 89-108. 38p. Vol. 72, Section B, No. 10: The Minor Acid Intrusions of the Aughrim-Ballinacash Area, Co. Wicklow. By J. G. Brindley and B. P. Connor. Pp. 165-184+plates 8 and 9. 26p. Vol. 72, Section B, No. 11: The Late-Glacial and Post-Glacial Mollusca of the White Bog, Co. Down. By A. W. Steffox, J. G. J. Kuiper, Nora F. McMillan and G. F. Mitchell. Pp. 185-208. 30p. (Dublin: Royal Irish Academy, 1972.) [107]

Building Research Station Current Paper 12/72: Demolition. By E. A. Akam. (Reprinted from *Civil Engineering*.) Pp. 4. (Garston, Watford: Building Research Station, 1972.) *gratis*. [107]

Memoirs of the Royal Astronomical Society. Vol. 76, Part 3: Photographic Measures of Double Stars. By J. McK. Luck. Pp. 67-98. Vol. 76, Part 4: The Earth's Acceleration as Deduced from Al-Biruni's Solar Data. By R. R. Newton. Pp. 99-128. (Oxford and London: Blackwell Scientific Publications, 1972. Published for the Royal Astronomical Society.) [107]

Scientific Instrument Manufacturers' Association of Great Britain. 55th Annual Report 1971-72. Pp. 22. (London: SIMA, 1972.) [117]

John Innes Institute. Sixty-second Annual Report, 1971. Pp. 133. (Norwich: John Innes Institute, 1972.) 50p. [127]

Administrative Justice and Supplementary Benefits. By Melvin Herman. (Occasional Papers on Social Administration, No. 47.) Pp. 68. (London: G. Bell and Sons, 1972.) £1. [137]

National Institute of Agricultural Botany. Fifty-second Report and Accounts 1971. Pp. 83. (Cambridge: National Institute of Agricultural Botany, 1972.) [137]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 272, No. 1226: The Motion of a Vortex Filament with Axial Flow. By D. W. Moore and P. G. Saffman. Pp. 403-429. 70p. £1.95. Vol. 272, No. 1227: A Kinematic Theory of Large Magnetic Reynolds Number Dynamos. By A. M. Soward. Pp. 431-462. 85p. £2.35. Vol. 272, No. 1228: Limit Analysis for Plates—a Simple Loading Problem Involving a Complex Exact Solution. By E. N. Fox. Pp. 463-492. 80p. £2.25. (London: The Royal Society, 1972.) [147]

Forestry in Wales. 34. (Cardiff: The Forestry Commission, 1972.) *gratis*. [147]

Entry to Chemistry Courses at the Tertiary Level: a Preliminary Survey. (The British Committee on Chemical Education. A joint committee of the Royal Society and the Chemical Society.) Pp. 13. (London: Education Officer, The Chemical Society, 1972.) 10p. [147]

UK Chemical Industry Statistics Handbook. Fourth revised edition. Pp. 111. (London: Chemical Industries Association Limited, 1972.) £4 (member companies £2). [147]

British Overseas Trade Board. Export Handbook: Services for British Exporters. Fifth edition. Pp. 197. *gratis*. Report of Task Force. Pp. 20. Initial Report 1972. Pp. 19. (London: British Overseas Trade Board, 1972.) [147]

London and Home Counties Regional Advisory Council for Technological Expansion. Bulletin of Special Courses: Short Advanced Courses in Technology, Management Studies and Commerce in the Region. 1972/73, Part 1, Autumn Term. Pp. 110. (London: London and Home Counties Regional Advisory Council for Technological Education, 1972.) 60p. [247]

Rothamsted Experimental Station. Report for 1971, Part 1. Pp. 405. Report for 1971, Part 2. Pp. 204. (Harpenden, Herts: Rothamsted Experimental Station, Lawes Agricultural Trust, 1972.) £2 the two parts. [247]

Critical Reviews in Tribology 1970. Pp. 100. (Guildford: IPC Science and Technology Press, Ltd., 1972.) £6; \$16.80. [247]

Building Research Station Current Paper 13/72: 'Social' Housing in Europe. By Evelyn Cibula. Pp. 5. (Garston, Watford: Building Research Establishment, 1972.) *gratis*. [257]

Ministry of Agriculture, Fisheries and Food. Technical Bulletin No. 23: Avian Embryo Development. Pp. vii+24. (London: HMSO, 1972.) £2.25 net. [267]

The Radcliffe Observatory, 1772-1972. By A. D. Thackeray. Pp. 56. (London: The Radcliffe Trustees, 1972.) [267]

The Gas Council. Research Communications. No. 180: Report on the Gas Council Research Scholarships, 1971. Pp. 8. 50p. No. 181: Recent Developments in Domestic Heating by Hot Water. By B. S. Cheyney and E. A. K. Patrick. Pp. 16. 50p. No. 182: A Study of the Performance of Automatic Packaged Gas Burners. By M. L. Hoggarth, D. A. Jones and K. F. Pomfret. Pp. 15. 50p. No. 183: 62nd Report of the Joint Refractories Committee, 1970-71. Pp. 24. £1. No. 184: The Prediction of Heat Transfer and Thermal Performance in Gas-Fired Processes. By R. M. Davies, D. M. Lucas and Mrs. H. E. Toth. Pp. 19. £1. No. 185: Some Characteristics of Practical Spark-Ignition Systems. By B. Ekins and J. R. Wilson. Pp. 14. £1.50. No. 186: Soot in Laminar Gas Flames. By Stuart B. Reed and Frank G. Roper. Pp. 17. £1. No. 187: The Properties of British Natural Gas. By A. B. Densham and R. M. Gibbons. Pp. 13. 50p. No. 188: Inspecting Inspectors. By R. Chrisp, R. F. Lumb, B. Phelps and R. B. Gibbon. Pp. 14. 50p. No. 189: A Review of Computer Programs for Network Analysis (Developed at London Research Station). By A. E. Fincham. Pp. 11. 50p. No. 190: Safety Shut-Off Systems for Gas Burners. By S. H. Hutt and K. Stein. Pp. 13. 50p. No. 191: Thermal Design for Fan-Assisted Balanced Flues. By N. C. Ross and J. C. Tipping. Pp. 15. 50p. (London: The Gas Council, 1972.) [167]

The Institution of Gas Engineers. Communications. No. 857: 32nd Report of the Chairman's Technical Committee, 1970-71. Pp. 16. 50p. No. 858: Second Report of the Education and Training Committee, 1970-71. Pp. 15. 50p. No. 859: Gas for Radiant Space Heating. By F. M. H. Taylor. Pp. 11. 50p. No. 860: A Study of Unvalved Pulse Combustors. By F. E. J. Briffa, P. W. Staddon, R. N. Phillips and D. R. Romaine. Pp. 12. 50p. No. 861: Fundamentals of Methane Combustion. By G. Dixon-Lewis, J. E. Garside, J. K. Kilham, A. L. Roberts and A. Williams. Pp. 9. 50p. No. 862: Institution Gas Research Fellowship Report. 1967-70. Luminous Emissivities of Natural-Gas Diffusion Flames. By A. J. Newall and T. M. Lowes. Pp. 11. 50p. No. 863: Recent Developments in Flueing. By J. Emerson and M. J. Randall. Pp. 17. £1. No. 864: Marketing Research in the Natural Gas Industry. By F. J. Johnson. Pp. 14. 50p. Discussions, 37th Autumn Research Meeting, London, 1971. Pp. 80. £1. (London: Institution of Gas Engineers, 1972.) [267]

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